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# THE CONCEPT OF COLLAGEN DISEASES*

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The term diffuse collagen disease was originally applied to acute and chronic maladies which are characterized anatomically by generalized alterations of the connective tissue, particularly by abnormalities of its extracellular components. In this sense the term can include rheumatic fever, rheumatoid arthritis, polyarteritis, acute lupus erythematosus, generalized scleroderma, and dermatomyositis. A critical consideration of the term is necessary today in order to ascertain whether its frame of reference is useful for further investigation and for the ultimate pathogenetic definition of that group of diseases to which it was originally applied.

The idea that the characteristic organ and tissue alterations in rheumatic fever and rheumatoid arthritis reflect a systemic involvement of the entire connective tissues of the human body was first proposed by Klinge.[1] While not the first observer, he focused attention upon the conspicuous changes of the intercellular components of the connective tissue, the fibrinoid alteration of the collagenous tissue and the myxomatous swelling of the ground-substance. The occurrence of similar connective tissue changes in rabbits made hypersensitive to foreign proteins (Gerlach,[2] Klinge[1]) led him to the conclusion that the tissue damage in human rheumatic disease was due to hypersensitivity. Consequently, Klinge[1] maintained that the same pathogenetic mechanism applied in other disease entities characterized anatomically by fibrinoid connective tissue damage. He therefore included periarteritis nodosa, dermatomyositis, malignant nephrosclerosis, thrombo-angiitis obliterans, certain nephritides, and cardiovascular sepsis (subacute bacterial endo-

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carditis). The observation of fibrinoid vascular lesions in scleroderma led Masugi and Yä-Shu[8] to include this disease among the allergic maladies. It was only logical that acute lupus erythematosus in which frequently, though not universally, striking fibrinoid tissue changes were observed, could be interpreted in the same way. Pollack, Baehr, and I[4] were fully aware of this possibility. However, we believed that such a sweeping biologic-anatomic synthesis was premature. We were cautioned by the fact that local fibrinoid collagen damage occurred in situations where the mechanism of hypersensitivity could be excluded, as in the base of peptic ulcers (Askanazy[5]) and in the vicinity of pancreatic necrosis. The observation of Wu[6] that trivial mechanical injury provoked fibrinoid collagen alteration in the skin of the rat also challenged the exclusive significance of hypersensitivity in its production. Moreover, we were impressed by the frequency of widespread vascular necrosis, simulating polyarteritis, in experimental hypertension. The recent observations of Byrom and Dodson[7] who produced fibrinoid arterial necrosis in normal animals by brief rises in intra-arterial tension seem to emphasize the significance of the mechanical factor. Identical vascular lesions had been produced by Winternitz *et al.*,[8] Holman,[9] Duff and associates,[10] and by Koletsky,[11P] as presented today, under experimental conditions in which hypersensitivity could be definitely excluded as a pathogenetic factor. Waters'[12P] new observation that repeated adrenalin injections produce vascular necrosis is of particular importance because it indicates that arterial lesions simulating human polyarteritis may depend on primary injury of smooth muscle. This might well be of significance in human pathology. These observations also illustrate that an apparent similarity in the histologic picture does not denote identity of tissue lesions and certainly not unity of process and pathogenesis. Holman's[13P] investigations reflect the same lesson and I shall return to their further significance at a later phase of my presentation. The previously cited experiences led us to the belief that the connective tissue changes demanded further analysis before the question of pathogenesis could be decided. From the preceding observations of Klinge[1] and his followers and our own findings in acute lupus erythematosus we were, however, convinced that a common denominator existed in the striking alterations of the extracellular portions of the connective tissue. Under the influence of the concepts of Schade[14] and of Standenath[15] and of the pathologic-anatomic tradition (Morgagni), we regarded the connective tissue as an organ which we designated as the common seat of these heterogenous maladies. Because of the conspicuous morbid manifestations of the extracellular components, we suggested the

term collagen diseases in a pars pro toto sense. I believe today that even this cautious synthesis was premature because it resulted in an indiscriminate acceptance of a term with a diagnostic and pathogenetic import not originally intended when it was conceived. We did not deny the probable rôle of hypersensitivity but we were interested in a search for all factors which might be responsible for the conspicuous structural alterations and, particularly, in the mechanism of their action. It was obvious that such an inquiry had to take its origin from the existing information regarding the structure and the biology of the normal connective tissue, specifically of its extracellular components.

Soon after the connective tissue cells had been discovered by Schwann,[16] the question arose as to the origin of the intercellular substances. In 1861 Kölliker[17] summarized the state of the problem as it was viewed by the majority of investigators of this period.

"In general and in principle," Kölliker wrote, "I agree with Virchow in conceiving of the fibrillar connective tissue as a mere filling portion and of the cells as the significant part. The fibrillar collagenous substance does not directly develop from cells. But the idea that the cells excrete the ground-substance doubtlessly conforms with the belief and concept of many modern histologists; however, I freely agree with Henle that this point of view is not supported by unequivocal facts, because the ground-substance might deposit itself independently. What is meant by the notion referred to above, is in fact not so much that the intermediary substance originates exclusively from the connective tissue cells, but that its chemically characteristic factor is under the direct influence of the cellular elements. I consider that, as in the secretion of a gland, one part of the material is derived from external import but another part from the activity of the cells. Thus, one might assume that mucus and collagenous substances which do not occur in the blood, are formed under the direct influence of cells. I even consider it possible that these substances are formed within the cells, then escape from them and subsequently consolidate."

I have quoted Kölliker so extensively because he clearly poses the problems which today still await an unequivocal answer: (1) the mode of fiber formation, (2) the nature and site of fabrication of the homogeneous ground-substance.

In the years following Kölliker's summary, prevailing opinion expressed itself in favor of his view that the intercellular substances are formed by the fibroblasts. However, observations directly proving this intracellular formation were never supplied and in fact the existence of such cellular activity was seriously challenged by Baitsell's original

tissue cultural and other studies,[18,19] and by Nageotte's experiments with wound healing.[20] Even long before these investigations, von Ebner[21] had demonstrated that collagen fibers develop in the chorda sheath of lower fish without the presence of mesenchymal cells.

What is the state of our knowledge today? Baitsell[18] in his classical investigations had concluded that fibrin was transformed into a fibrous tissue which in its morphologic structure, to say the least, is apparently identical with normal connective tissue of the frog. Because the newly formed fibers were not resistant to trypsin, he conceded that the ultimate proof of the transformation of fibrin into collagen was still lacking. In 1949 Porter and Vanamee[22] demonstrated that the fibers formed in cultures of chick-embryo skin and foregut, rabbit thymus, and rat pericardium show the characteristic periodicity of collagen fibers[23,24] under the electron microscope and that they resist trypsin digestion. While these experiments prove the collagenous nature of the fibers in tissue culture, the rôle of the fibroblasts in their formation has not been ultimately decided, although Porter and Vanamee stated that the fiber is apparently not spun off the cells. While Baitsell[25] believed that fibrous tissue could be formed from a plasma clot by mere mechanical factors without cellular action, Doljanski and Roulet[26] maintained that the "fibril-forming process always is the result of a direct interaction (*Fühlungsnahme*) between cell and surrounding plasmatic environment." Hass and McDonald[27] also expressed the belief "that fibroblasts perform an indispensable rôle in collagen formation" in tissue culture. They showed that cessation of fiber formation was generally associated with morphologic evidence of early fibroblast degeneration, but that "a depression of the pH of the medium to lower limits which permitted continued growth and viability of fibroblasts was usually correlated with a cessation of collagen deposition." These observations illustrate the reciprocal relation between fibroblast and surrounding medium controlling the mechanism of fiber formation and led to their conclusion that "normal fibroblasts and normal mediums interact to yield collagen *in vitro*."

I have dealt at this length with the *in vitro* formation of collagen fibers because the situation of fibroblasts within culture medium can be likened to the conditions of the connective tissue in the living. Indeed, Doljanski and Roulet[26] considered the intercellular substratum as the ground-substance of the culture analogous to the homogeneous ground-substance of the connective tissue *in vivo*. This point of view is of significance because it conforms with the idea of Nageotte,[20] who maintained that the fibroblasts in the animal body do not produce the intermediary substance

which is furnished rather by the body fluids. Certainly information gained from experimental variations of the culture medium, such as studies of cell cultures in synthetic media (A. Fischer,[28] and Morgan, Morton, and Parker[29]) and particularly of tissue culture in plasma of patients with various diseases, could advance our knowledge of the connective tissue in normal and morbid states. Investigations of the simpler system of tissue culture are more likely to clarify the problem of normal and abnormal fiber formation than those of the complex conditions of the living animal. Yet, investigations of the living animal may result in observations which do not correlate with those reached with the method of tissue culture. Hass and McDonald[27] found that low ascorbic acid values had no influence on collagen production in the isolated system of the tissue culture. Their findings contrast with those of von Jeney and Törö[30] who came to the conclusion that collagen fiber formation was promoted by the addition of ascorbic acid to the culture medium.

Wolbach and Howe[31] are credited correctly with the important discovery that ascorbic acid is essential for the formation of collagen fibers; but the process involved has not yet been fully clarified (Reid[32]). Wolbach and Bessey[33] tentatively suggested that the mode of action by which ascorbic acid promotes the formation of collagen "may be involved in the chemical mechanisms (enzymes) of the cells responsible for the synthesis of this protein product." Danielli, Fell, and Kodicek[34] believed that ascorbic acid deficiency affects the fibroblasts which show a reduced degree of phosphatase activity. On the other hand, Penney and Balfour[35] recently reported that there is a failure in the production of acid mucopolysaccharides in the wounds of a guinea-pig depleted of vitamin C. These observations have been confirmed by our own investigations. On the other hand, Gersh and Catchpole,[36] in a more recent publication, stated that there is an increased amount of ground-substance in scorbutic guinea-pigs. They stressed the fact that much of this ground-substance consists of alcohol-insoluble, water-soluble glycoproteins. The latter are, in their opinion, in a state of depolymerization. The differences in the amount of ground-substance in these two sets of experiments may depend on the difference in staining technics used. The results need not be considered as incompatible, inasmuch as both observations reach the conclusion that the ground-substance is abnormal in scurvy. Both tend to show that not only normal fibroblasts but also a homogeneous ground-substance, adequate in amount and in chemical or physicochemical constitution, is of paramount importance for the formation of collagenous fibers. The fact that changes in ground-substance and collagen fibers are so striking in the so-called collagen

diseases makes it evident that a fuller understanding of alterations in the elaboration and constitution of these materials is needed to clarify their pathogenesis.

One is intrigued also by the question as to the mode of action of the adrenal cortex and other hormones upon the formation of collagen fibers. The investigations of Taubenhaus and Amromin[37] have shown that desoxycorticosterone stimulates fibroblasts and encourages the deposition of collagen around sterile abscesses, while testosterone and estradiol inhibit fibroblastic response and collagen formation. The investigations of the New York Presbyterian Hospital Group[38P] and of Spain and associates[39P] have shown cortisone to have a marked inhibitory effect in experimental wound healing. I should like to refer also to the inhibition of anaphylactic[40] and anaphylactoid[41] tissue reactions by ACTH. On the other hand, Baggenstoss[42P] has demonstrated a marked fibrosing effect of cortisone in patients with polyarteritis. In one case of acute lupus erythematosus treated with ACTH, I could still see acute fibrinoid alterations of the connective tissue, while in another case there was increased fiber formation in the heart, but the fibers seemed to be abnormal because of irregularity in width and staining quality. It should be added that in both cases "L.E." cells[43] were persistently found during life and hematoxylin bodies[44] were present in several organs post mortem. These divergent experimental and pathologic-anatomic observations are difficult to interpret. The inhibition of fibroblastic response seems to indicate a direct influence of the adrenal hormones upon the cells. But the experience with tissue cultures shows that fiber formation is the result of interaction between fibroblasts and medium. By analogy, a possible action of the hormones directly upon the formation of the extracellular substances or upon their chemical reactivity and physical state also must be taken in consideration. I shall return shortly to some observations which point in that direction.

The mucinous nature of the homogeneous ground-substance has been known for decades (Rollett[45]). The investigations of Karl Meyer[46] and his associates have greatly advanced our knowledge of the mucopolysaccharides which enter into its constitution. They are mainly hyaluronic acid and chondroitin sulfuric acid. The occurrence and influence of depolymerizing enzymes (hyaluronidase) and their relation to the spreading factor of Duran-Reynals[47] has been clarified by Meyer and others. The development of specific inhibitors and the occurrence of a non-specific antihyaluronidase have been demonstrated by Glick.[48] The rôle of such inhibitors is equal in importance to that of hyaluronidase under normal and abnormal conditions. The opposite effects of luteinizing and follicular hormones upon the spreading of dyes in the rabbit

skin (Sprunt and McDearman[49]) and the inhibition of hyaluronidase by desoxycorticosterone (Seifter *et al.*[50]) demonstrated the influence of hormones upon the interaction between hyaluronic acid and hyaluronidases. The presentation of Rinehart and Greenberg[51P] shows that pyridoxine deficiency causes a striking deposition of metachromatic substance in the arterial wall.

It is generally accepted that the mucopolysaccharides are linked to proteins within the ground-substance (Ropes *et al.*[52]). Very little is known about the chemical constitution of the protein moiety and its modifications under normal and abnormal conditions. It is digested by trypsin, as experiments of Day[53] have shown. That it contains tyrosine is indicated by the fact that the xanthoprotein reaction of connective tissue is abolished after removal of the ground-substance by baryta water (Rollett[45]). Ogston and Stanier[54] recently reported that synovial fluid contains hyaluronic acid in close association with protein; the latter constitutes about 30 per cent of the complex. The amino acid partition was studied by paper chromatography.

From this very superficial review of the chemical nature and of certain physiologic factors concerning the ground-substance, it is obvious that microscopic investigations of its structure in normal and abnormal conditions are of paramount importance. This task is restricted by technical difficulties partly inherent in the easy solubility of the mucopolysaccharides in the conventional fixatives. Preparation by freezing and drying is obviously the ideal technic but unfortunately it cannot yet be applied routinely in the investigations of pathologic-anatomic material. However, the examination of the connective tissue in the disorders under consideration has revealed conspicuous alterations of the homogeneous ground-substance. Klinge[1] referred to its myxomatous swelling in rheumatic fever and I[55] have stressed the same feature in acute lupus erythematosus and have demonstrated an intense toluidine blue metachromasia, which was abolished by preceding treatment of the sections with bull-testis hyaluronidase.

Such morphologic observations urge upon the medical investigator the desire to understand the mechanism by which these structural alterations are provoked in disease. He tries to correlate his observations with the facts known to chemists and physiologists, but he soon realizes that certain fundamental problems regarding the nature and origin of the homogeneous ground-substance are as obscure as in the days of Kölliker.[17] Vaubel[56] has studied the problem of intracellular mucin formation in tissue cultures of synovial cells and of fibroblasts. He found mucin in the supernatant fluid of cultures of the former, but no mucin in fibroblast culture medium. In Wolbach's[57] classical experi-

ments the appearance of vacuoles in fibroblasts and the accumulation of a non-fibrillar intercellular substance pointed to the fibroblasts as the manufacturers of this material. But the reinvestigations of the developing granulation tissue in scorbutic guinea-pigs by Penney and Balfour[35] revealed fat within these vacuoles and a failure in the production of mucopolysaccharides. We have made similar observations.

Moreover, in experiments by Ludwig and Boas,[58] designed to study the rôle of hormones in the deposition of ground-substance, newborn chicks have been injected with testosterone. Relatively large amounts of metachromatic substance appeared in the comb within 12 days, while control birds did not show significant amounts of mucopolysaccharides. While the fibroblasts were conspicuously large and showed cytoplasmic basophilia and phosphatase activity, no metachromatic material could be detected within them. The metachromasia of the ground-substance was abolished by preceding treatment of the sections with bull-testis and streptococcal hyaluronidase, and Boas[59] extracted large amounts of hyaluronic acid from the cock's comb. It should be added that the injection of estrogen, adrenal cortex extract, and of cortisone did not cause deposition of metachromatic ground-substance.[60]

In another series of experiments Ludwig, Boas, and Soffer[61] treated thyroidectomized guinea-pigs with thyrotropic hormone. They all developed exophthalmos. Within the connective tissue of the orbital structures a distinct increase in the amount of metachromatic material was noted. Identical changes were seen in the retroperitoneal tissues. Chemical examination revealed a rise in hexosamine content of these tissues with an increase in water content and an elevation of serum hexosamines. These observations again demonstrated that the deposition of mucopolysaccharides is under hormonal control, a fact well known from experience with myxedema and with pretibial myxedema in hyperthyroidism (Watson and Pearce[62]), and from experiments of Selye,[63] Ogston and associates,[64] and others. Yet neither our experiments nor those of others brought forward conclusive evidence that the mucopolysaccharides are formed by fibroblasts. On the other hand, Gersh and Catchpole[36] in a recent paper showed the presence of granules positive to the periodic-acid, sulfurous-fuchsin technic in fibroblasts in situations where ground-substance is laid down. They interpreted these granules as glycoprotein and expressed the belief that their observations proved a secretion of ground-substance by fibroblasts. Obviously their conclusions rest on chemical identification by the McManus stain. From what we have learned from McManus[65P] today it seems to me that he would not go that far, and that his only claim regarding chemical identification is that substances stained by his method contain carbohydrates.

In view of the great diversity of tissue components and other substances stainable by the technic, great caution must be exercised in the dynamic interpretation of microscopic pictures. However, the paper by Gersh and Catchpole[36] contained other observations which are of great importance. Because these refer to variations in stainability of the same reactive material before and after extraction with water, such differences cannot depend on chemically different substances. Gersh and Catchpole believed that such variations are determined by changes in polymerization of the glycoproteins of the ground-substance. It would be highly desirable to apply the same technic of water extraction to pathologic material in order to analyze the nature of the changes in the ground-substance which are so conspicuous in rheumatic fever and acute lupus erythematosus. It is evident that neither the chemical nature of the ground-substance nor the site of its manufacture is sufficiently clarified.

An abnormality of the ground-substance might be determined by a disturbance of cellular enzymatic activity *in situ*. For instance Janeway[66] recently has suggested that antibody antigen union in sensitized animals could lead to the release of enzymes from connective tissue cells. But it is also conceivable that abnormality of the ground-substance is correlated with abnormality of the blood plasma, which necessarily provides the chemical building blocks for its formation. This possibility is suggested by our experiments with thyrotropic hormones and by the fact that in acute lupus erythematosus, with its increase in mucinous ground-substance, the hexosamines are always strikingly elevated in the blood serum. It is of significance that they are decreased by treatment with cortisone and ACTH. Further investigations correlating serum hexosamines and the amount and quality of mucopolysaccharides in connective tissue certainly are needed. In reference to the protein moiety of the ground-substance, Coburn and Moore[67] and Teilum[68] expressed the belief that glomerular capillary changes in acute lupus erythematosus could be the result of a precipitation of globulins which are so persistently elevated in this disease. The conspicuous changes in the serum proteins in this disease are distinctly influenced by cortisone and ACTH. Miss Reiner, in our laboratories, found that serum-albumin levels rise, while gamma globulins are decreased under cortisone and ACTH treatment. The alpha 2 globulin partition, however, which is also elevated, remains unchanged. Holman's[18P] investigation seems to point also in the direction that some change, not yet defined, of the blood plasma could be responsible for necrotizing alterations of the vessel wall. Parenthetically, mention should be made of the recent report of abnormal lipoprotein macromolecules in the blood plasma of arteriosclerotics.[69] Haserick's[70P] discovery of an abnormal antigenic constituent of the plasma

in acute lupus erythematosus, associated with the gamma globulins, adds further evidence for the importance of blood plasma changes in the pathogenesis of one of the members of the group. The inclusions of the "L.E." cells have recently been shown by Lee and associates[71] in our laboratories to contain depolymerized desoxyribose nucleic acid, similar to the hematoxylin-stained bodies in the tissues of acute lupus erythematosus. Haserick's factor is therefore apparently responsible for this peculiar disturbance of nucleic acid metabolism of mesenchymal cells. Its relation to the changes in the intercellular substance in acute lupus erythematosus is not clear, although the association of nuclear changes with fibrinoid alterations of connective tissue is sometimes striking in vessels and in the heart.

The fibrinoid collagen change has always dominated the discussion of the structural alterations of the group referred to as collagen diseases. In fact, it has been singled out as the basic principle for pathogenetic explanation. I do not want to repeat my reasons for not accepting the thesis that fibrinoid alteration of collagen in human disease is pathognomonic for hypersensitivity. More information is needed regarding the composition of the substance referred to as fibrinoid before we indulge in biologic-anatomic correlations. The term as originally applied by Neumann[72] referred to collagen fibers which assume the structural and tinctorial quality of fibrin. This alteration of the fibers was believed to be the result either of an impregnation of the intact or degenerated collagen fiber with fibrin or of a disintegration of the collagen fiber without additional fibrin impregnation (Ricker,[73] Bahrmann[74]). Klinge[1] spoke of a transformation of the connective tissue substance with the formation of waxy, highly refractive masses and believed that fibrinoid degeneration of connective tissue is the result of swelling and chemical alteration of the ground-substance. Altshuler and Angevine[75] also believed that the ground-substance is the only constant anatomic element in the formation of fibrinoid and that it is formed by precipitation of the acid mucopolysaccharides. They assumed that the precipitant is probably an alkaline protein derived from tissue necrosis or the interaction of the tissue with a damaging agent. These conclusions are of great interest because they indicate that abnormal fibrils may be formed in the ground-substance instead of the typical collagen fibers. Is it not permissible also to assume that a primary abnormality in chemical constitution of the ground-substance due to imperfect formation might be responsible for the appearance of abnormal fibers with the characteristics of fibrinoid? These are merely working hypotheses which should be pursued by further investigations. Again, tissue cultures with well designed variations of the nutrient media might clarify the problem.

But the constitution of the fibrinoid material could also come under direct attack with methods other than those of conventional histopathology. The presentation of Gross[76P] and his previous work have shown the intrinsic value of the electron microscope for the identification of the ultrastructure of the normal collagen fiber. Application of this fundamental method to the connective tissue in morbid states, as shown by Gale,[77P] is certainly most desirable. One is aware that such difficult investigations will not mature for a long time and will require further advances of technic, such as microsections. We have used x-ray diffraction in the examination of subcutaneous nodules of rheumatoid arthritis and endocardial vegetations of acute lupus erythematosus. The pattern obtained was that of fibrin, but x-ray diffractionists inform me that the term fibrin-pattern covers substances of a wider range than fibrin only. I believe that the methods described by Hass[78P] for the analysis of amyloid could well be applied to the investigation of the same material.

When we originally proposed the term collagen diseases we were aware that the structural alterations which had been disclosed by the conventional technics of microscopic anatomy required further analysis. We believed that the visible alteration of the intermediary substances was only a manifestation of a profound disturbance of their chemical and physical constitution. We recognized that the morphologic observations did not constitute a pathogenetic definition of the maladies grouped together and that the term did not imply identifying them with one another. We realized that a rational inquiry into the pathologic states of the collagenous material must be based upon a full comprehension of its normal constitution and biology. We did not fully realize the depth of this problem at that time. I have tried to outline today not only how fragmentary our knowledge still is but also how rapid is the tempo of new advances. The fundamental question of fiber formation will be brought to a solution by tissue culture and electron microscopy. The chemical nature of the homogeneous ground-substance, its origin, and the influence of enzymes and hormones upon its constitution are under vigorous scientific attack. A never-relaxing concentration upon these problems has convinced me that the concept of collagen diseases is not an idle speculation. The increasing interest of the medical profession could support me in my conviction; but the impatience of clinical investigators and a peculiar worship of diagnostic terms have led to an exaggerated popularity of the diagnosis collagen disease. There is danger that it may become a catch-all term for maladies with puzzling clinical and anatomical features. It is not a term applicable to diagnosis and certainly does not define the morbid process of the diseases grouped together. All we wanted to express originally was that in certain diseases

anatomical investigations reveal conspicuous alterations of the intermediary substances of the connective tissue in a systemic manner. Today, in selecting the title "Concept of Collagen Diseases" I took advantage of the definition of the word concept, which means an idea that includes all that is characteristically associated with or suggested by a term. In the present uncertainty of our knowledge regarding the origin of the intermediary substances and of the factors which regulate their structure as well as chemical and physicochemical constitution, it seems obvious that alterations of this material cannot be regarded as maladies of these substances or of the connective tissue as a whole. These morphologic disorders are only outward manifestations of morbid processes, the site and nature of which are still obscure. When further basic research has clarified the factors which control the plasticity of the connective tissues under normal and abnormal conditions, the concept of collagen disease will well have served its purpose.

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# NEURILEMMOBLASTOSIS

## The Influence of Intrinsic Factors in Disease When Development of the Body Is Abnormal*

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In a paper on subdural hemorrhage, cysts, and false membranes (Inglis[1]), changes were described which were thought to illustrate the influence of intrinsic factors in disease when development of the body is normal. The present contribution is concerned with the influence of intrinsic factors in disease when development of the body is abnormal.

The opinions expressed in this paper are submitted as a hypothesis. It is recognized that the evidence presented in their support is not conclusive, but it is thought that it provides a reasonable explanation of the relationship to one another of the various lesions that may be met with in patients suffering from "tuberous sclerosis" of the brain, and also of such lesions occurring in patients who present no clinical evidence of cerebral disease.

It is suggested that specific nerve sheath tissue is of outstanding importance to the subject under discussion. Opinions still differ as to the nature of nerve sheath tissue, but in the present paper the terms specific nerve sheath tissue, neurilemma, and sheath of Schwann are used synonymously.

### Definitions

*Neurilemmoblastosis* is used to describe a condition characterized by lesions, many of which are distinguished by the presence of cells (some of them primitive) regarded as akin to those that form the neurilemma or sheath of Schwann. In some of the lesions of neurilemmoblastosis distinctive cell groups cannot be identified; nevertheless, it is suggested that in the causation of such lesions the neural intrinsic factor of neurilemmoblastosis plays an essential part.

*Glioneurilemmoblastosis* is suggested as a suitable name when the brain is affected as well as other parts of the body.

*Neurilemmoblastoma* is used when the specific tissue of neurilemmoblastosis forms a tumor mass. Such a tumor is benign.

*Liponeurilemmoblastoma* is the name given to a tumor composed of a mixture of neurilemmoblastic tissue and lipomatous (adipose) tissue. This tumor also is benign.

*Neural intrinsic factor* is used to mean a specific factor, of intrinsic nature and neural origin; this factor is considered to underlie all of the

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lesions of neurilemmoblastosis. Neurilemmoblastosis is looked upon as a visible, recognizable, demonstrable manifestation of neural intrinsic factor. Neural intrinsic factor is considered to underlie other conditions, including neurofibromatosis with which neurilemmoblastosis has features in common; there are, however, important differences between these two conditions.

*Basic intrinsic factor* requires further explanation. The laws of growth and development operate through intrinsic factors from the time of fertilization of the ovum, and it is only after neural tissue appears in the developing embryo that neural intrinsic factor as such can come into play. For the more fundamental operation of intrinsic factors the term basic intrinsic factor is employed. Basic intrinsic factor is not regarded as apart from neural intrinsic factor, but as more primitive and comprehensive, and as embracing a potential neural intrinsic factor component. Neural intrinsic factor, on its more primitive side, merges into basic intrinsic factor; the line of distinction between them is indefinite, and it is often a matter of doubt as to whether the link between certain related pathologic conditions is at the level of neural intrinsic factor or of basic intrinsic factor.

CASE I

The patient (P.U.S. 110), a married woman, 41 years of age, had consulted a doctor for "asthma" 8 years prior to her death. She had suffered from attacks of breathlessness during the last 3 years of her life. A nodular rash on the nose and cheeks had been present since the age of 3 months. No mention of fits or of mental abnormality was made in the notes. She had had 4 children. Five days before death there was severe pain in the chest.

Dyspnea and cyanosis were found on examination. "Adenoma sebaceum" was present on the nose and cheeks. The left nipple was retracted and fluid was draining from it. The Wassermann reaction was negative; red blood cells, 6,850,000; hemoglobin, 141 per cent. The urine was acid and the specific gravity 1016; albumin, granular casts, and a few leukocytes and red blood cells were present; blood culture was sterile. Roentgenologic examination showed the right lung to be about half collapsed; the left lung showed mottling; the pulmonary artery was enlarged. The provisional diagnosis was Ayerza's disease.

*Autopsy Report*

At gross examination, the *thymus* was not recognizable. The *right lung* was collapsed as the result of pneumothorax due to rupture of an emphysematous bulla. Most of the collapsed lung was solid with blood, but the apex presented a striking picture of bullous emphysema. Dotted throughout the substance of the lung were white nodules about 1 mm. in diameter. The entire *left lung* was emphysematous. White nodules 1 to 2 mm. in diameter were present in the lung tissue. The most strik-

ing feature in the *heart* was enlargement of the pulmonary artery; it was larger than the aorta. The *liver* was enlarged and showed chronic venous congestion; in the right lobe were a few small whitish nodules about 1 mm. in diameter. The *spleen* showed no special changes. The *right kidney* weighed 515 gm. The capsular surface was studded with raised yellowish tumors. A large thin-walled cyst was present near the lower pole. In the small amount of capsular surface free from tumors a few small cysts could be seen. The cut surface showed small amounts of kidney substance free from tumors. The tumors involved medulla as well as cortex. Cysts were inconspicuous on the cut surface. The pelvis and ureter were not dilated. Most of the tumors were yellow, some were white, some were a mixture of yellow and white. The *left kidney* weighed 320 gm. (with adrenal attached). It was very similar to the right, but was slightly less involved by tumors. On the capsular surface, in the areas free from tumorous tissue, a few cysts were to be seen. The cut surface showed an appreciably larger amount of kidney substance free from tumors than did that of the right kidney. In the left kidney also, yellowish and whitish tumors were present, but for the most part the two types were blended. The pelvis was not dilated. In the portions of kidney which were free from tumors the capsular surface was smooth, and the cut surface showed that the cortex was not diminished in amount in relation to the medulla. The *uterus,* when cut into, was seen to contain small nodules similar to those found in the liver and lungs. The *esophagus, stomach, intestines, adrenal glands, pancreas,* and *ovaries* appeared normal. The *brain* and *spinal cord* were not examined.

## Histologic Findings

The term malformation might be applied, perhaps more correctly, to some of the lesions referred to in this paper as tumors or neoplasms, but it has been found impossible to draw a line of demarcation between the different stages of the pathologic process, because areas of abnormal tissue which appear to show malformation merge insensibly into others which appear to be tumorous or neoplastic.

*Kidneys.* Three varieties of renal lesions call for special comment: white tumors, yellow tumors, and cysts. Histologically, it was found that the white tumors were composed mostly of elongated and round cells, and that at least a few fat cells were present in most of them. The yellow tumors, though composed mainly of adipose tissue, contained scattered areas of elongated and round cells.

*White Tumors.* The tissue composing the white tumors is considered to provide a key to the interpretation of many of the changes in the organs and tissues of the body outside the central nervous system in patients suffering from "tuberous sclerosis" of the brain. A common appearance of this cellular tissue is to be seen in Figure 1 in which the picture is thought to resemble that seen when neurilemmal cells proliferate after division of a nerve, or that to be seen in a spontaneous schwannoma (neurilemmoma). A striking feature of the cellular tissue in these renal tumors was the great variation in the size and shape of the cells. In Figure 1, leashes of spindle cells are to be seen passing in various directions; the nuclei tend to be elongated and to have rounded ends. Speaking generally there was little tendency to "palisading" in the cellular tumors, no more than is to be seen in Figure 1. Another appearance is to be seen in Figure 2 in which some of the nuclei are elongated and have more or less parallel sides, their ends being blunt and sometimes almost square. In many situations the cells were rounded rather than elongated, and sometimes were much smaller than the elongated cells (Fig. 3). Parts of the cellular growths were very vascular (Fig. 4). Here the vascular channels were large and their walls varied in thickness; some of the walls were hyaline and continuous with similar hyaline bands in the main tumor masses. The hyaline material stained green with Masson's trichrome stain. Spindle cells passed between the vessels and sometimes had a "perithelial" arrangement. Fat cells were often scattered among the spindle cells. This angiomatous appearance is thought to be due to the effect of the neural tissue on the blood vessels.

*Yellow Tumors.* The yellow tumors were composed for the most part of adipose tissue (Fig. 5) and large portions of them were composed solely of adipose tissue. The cells of the adipose tissue were indistinguishable from those of ordinary lipomata or of normal subcutaneous adipose tissue. Many of the fatty tumors were not clearly defined at the periphery; indeed, a striking feature was the way in which single fat cells, or clusters of fat cells, appeared isolated, either in the cellular tumors or between renal tubules where no other neoplastic tissue was to be seen. In most of the fatty tumors there were collections of tumor cells like those which formed the white tumors.

*Cysts.* The large cyst in the right kidney was probably of the same nature as the smaller ones in the parts of both kidneys which were free from tumors. In Figure 6 one of these cysts is to be seen. It may well be related to congenital polycystic disease of the kidneys, but Figure 6

does not provide in itself convincing evidence of this relationship. Stronger evidence that the renal cysts found associated with neurilemmoblastosis are part of the polycystic disease complex is provided by case 2.

*Uterus.* A general impression of the structure of the uterus in case 1 is given by Figure 7, which shows the cut surface of a portion of the myometrium, including part of the peritoneal surface. The myometrium is dark, the tumor nodules pale. On the left there is a large wedge-shaped tumor nodule (A) with base at the peritoneal surface, at the top of the figure a rather broken-up nodule, and in the lower part of the figure several smaller nodules, one indicated by the letter B. The structure of the largest nodule (A) is to be seen under higher magnification in Figure 8. Here the lower third (which is dark) represents myometrium, and the upper two-thirds (pale by contrast) is composed of ill defined spindle-shaped cells and many small structureless, somewhat homogeneous areas which stained green with Masson's trichrome stain and are thought to be akin to similar areas in some of the renal tumors (cf. Fig. 8 with Figs. 1 and 4). The tumor shown in Figure 8 is thought to be of neural origin like those in kidneys and lungs, and to be part of the widespread condition of neurilemmoblastosis. Even the smallest neoplastic foci in the uterus were pale by comparison with the adjacent myometrium, whereas very small myomata (in sections stained by hematoxylin and eosin) are usually darker than the adjacent myometrium because of their large number of involuntary muscle cells with blue-stained nuclei closely packed together. Another point of interest was that the small neoplastic foci in the uterus of case 1 (like many neoplastic foci in other organs in this case) showed blurred cellular definition, whereas in very small uterine myomata the cells were generally clearly defined.

*Liver.* The liver showed conspicuous venous congestion and a few small tumor nodules. One of the nodules is illustrated in Figure 9. It was in the substance of the liver, but not far beneath the capsule. The nodule was composed of adipose tissue, and apparently of adipose tissue alone. No fat was present in the epithelial cells of the hepatic parenchyma. The lipoma was not encapsulated or circumscribed. The isolation of fat cells is thought to be due, not to infiltration from the main mass, but to transformation of ordinary connective tissue cells into fat cells *in situ*. Similar appearances were seen in the kidney. Likewise when so-called congenital rhabdomyoma forms in the heart, the tumor is not encapsulated or circumscribed, but shows cardiac muscle cells, separate from the main tumor mass, presenting an early stage of the characteristic changes *in situ*. Figure 10 shows interesting changes in

a portal tract. A bile duct, indicated by the letter A, is seen in the lower part of the portal tract, and a portion of a dilated branch of the portal vein in the upper part; below, and to the left, hepatic parenchyma surrounds the portal tract. The enlargement of the portal tract is due to a mixture of two elements, ill defined spindle-celled tissue and adipose tissue in which the fat spaces are of different sizes. This composite picture is regarded as similar to the mixture of neural cellular tissue and adipose tissue to be seen in many of the renal tumors. In Figure 11 changes similar to those shown in Figure 10 are seen, but at a somewhat earlier stage. The abnormal zone in the center is surrounded by parenchymatous tissue, only slightly engorged, and therefore probably peripheral so far as lobular arrangement is concerned. The central abnormal zone is therefore probably an enlarged portal tract with a dilated branch of the portal vein in the center. The rounded spaces are thought to indicate fat cells, and the rest of the ill defined tissue partly cellular tissue of neural origin. The central area (Fig. 12) taken alone would be difficult to interpret, but, in the light of evidence revealed by Figures 10 and 11, it is regarded as a group of cells of neural origin akin to the spindle cells shown in the portal tract included in Figure 10, and of the same order as the cellular nodules in other parts (cf. Fig. 12 with Figs. 13 and 15).

*Lung.* The sections taken from solid portions of lung were especially interesting because they showed two outstanding appearances: the more or less circumscribed areas which were almost free from pigment, and the intervening zones in which pigment was abundant. The pigment gave the reactions for free iron, and was regarded as hemosiderin. The pigment-free areas were thought to be composed largely of neurilemmoblastic tissue; the pigment-containing areas were thought to be areas of collapsed lung, the hemosiderin in the lumina of the compressed air sacs being due to polycythemia and chronic venous congestion. This interpretation is thought to be in keeping with the appearances to be seen in Figure 13 which reveals in the lower part collapsed air sacs with thickened walls and blood pigment (hemosiderin) in the lumina, and in the upper part a nodule of tumorous tissue with somewhat blurred histologic detail, such as is common not only in the tumors of the lung but also in tumors elsewhere in the body (cf. Fig. 13 with Figs. 12 and 15). Another pulmonary tumor nodule is seen in Figure 14. The irregularly distributed hyaline bands in this nodule resemble those in some of the renal tumors (cf. Fig. 14 with Fig. 4).

*Lymph Node.* Several lymph nodes situated near the trachea were saved for histologic examination; these were slightly larger than normal

and contained collections of cells (some elongated, some rounded) considered to be essentially the same as those in tumor nodules in kidney, lung, and liver. One of these collections of cells (of the rounded variety) is included in Figure 15 (cf. Fig. 15 with Figs. 12 and 13).

*Skin of Face.* One of the nodules of the skin of the face was examined microscopically. No special changes were observed in the sebaceous glands, except for slight hyperplasia, but deviation from the normal was seen in the dermis where dilated vessels with thin walls were conspicuous and collections of lymphocytes also were present. The feature of the dermis, however, to which special significance is attached, was the presence, near the dilated vessels, of ill defined tissue in which spindle-shaped nuclei and structureless material were seen. This cutaneous lesion is thought to have a neural basis and to link with the nodules in kidney, lung, and other parts.

*Breast.* Sections of the breast showed an intraductal papilloma, the processes of which had a connective tissue core and an epithelial covering, the appearances corresponding with those commonly seen in hyperplastic cystic disease of the breast. This lesion may have been a chance accompaniment, but since Batchelor and Maun[2] mentioned tumors of the breast as among the many conditions that may be found in patients with congenital rhabdomyoma (congenital glycogenic tumor) of the heart, and since these cardiac tumors are commonly associated with tuberous sclerosis of the brain, the possibility of neural intrinsic factor (or basic intrinsic factor) having influenced the development of the mammary lesion in case 1 seems worth bearing in mind.

Congenital rhabdomyoma (congenital glycogenic tumor) of the heart, which is commonly associated with tuberous sclerosis of the brain, was not present in case 1, but it was present along with tuberous sclerosis of the brain and congenital polycystic disease of the kidneys in case 2.

### CASE 2

The patient (P.U.S. 5083), a male child, 4 months old, was said to have appeared normal at birth, but to have suffered from occasional screaming attacks. A week prior to admission he began to have twitching and rolling of the eyes.

### *Autopsy Report*

There were no external abnormalities. The *brain* showed the characteristic changes of "tuberous sclerosis" (Dr. R. D. K. Reye). In the *heart,* numerous small, pale yellowish, opaque areas were seen beneath the pericardium and endocardium, and larger, pale yellowish, firm tumors projected from the pericardial surface and into the cavities of the heart. The largest of these measured 2.7 by 2.1 by 0.7 cm. The *right*

*kidney* weighed 50 gm. and was composed, for the greater part, of large thin-walled cysts filled with clear fluid. A small portion of more solid tissue, honeycombed by cysts, occupied the lower pole. The *left kidney* closely resembled the right in appearance, and weighed 48 gm. No lesions were found in other organs.

### Histologic Findings

*Kidneys.* Cysts were present in both renal cortex and medulla. There was no significant increase in connective tissue. In Figure 16, microscopic cysts, into which glomeruli project, constitute a striking feature. This is commonly seen in polycystic disease of the kidney. Other cysts were larger and had no glomeruli projecting into them.

*Heart.* There were many separate nodules in the heart, which were not circumscribed. The cells composing the nodules were large, and in the cytoplasm of many of them conspicuous spaces were present. Near the main nodules an early stage of the process was revealed by the presence of small spaces in cardiac muscle fibers, the appearance resembling that seen in sections of fetal heart muscle at an early stage of development. The congenital rhabdomyomas of the heart are interpreted as due to an anomaly of growth and differentiation, the heart muscle fibers growing large but presenting an undifferentiated appearance. The small isolated lesions separate from the main nodules in the heart of case 2 are thought to be due to change *in situ* and not to extension from the main nodules.

## Localized Neurilemmoblastosis

Neurilemmoblastosis is regarded as a systemic disease which may manifest itself in many lesions in many organs, as in case 1. On the other hand, it is suggested that essentially the same disease may be in play when only one organ is affected, and two such examples of localized neurilemmoblastosis will now be described, namely, case 3 (liponeurilemmoblastoma of the kidney) and case 4 ("honeycomb lung," "cystic disease of the lung").

### LIPONEURILEMMOBLASTOMA OF THE KIDNEY

#### CASE 3

The patient (P.U.S. 4929), a woman, 56 years of age, complained of a lump in the left lumbar region, which was increasing in size. There were no urinary symptoms, and kidney function on the left side was good according to dye excretion. The tumor mass, together with the kidney in which it was growing, was removed surgically.

The specimen showed that an almost spherical tumor, approximately 13 cm. in average diameter, apparently had grown in the middle zone of

the kidney, leaving intact only the upper and lower poles, which appeared normal (Fig. 17). The tumor was circumscribed and appeared to be encapsulated. The cut surface presented a mottled appearance, yellow, white, and red areas being present.

### Histologic Findings

The yellow areas consisted predominantly of adipose tissue, but collections of elongated cells were present to some extent. The white areas were predominantly cellular, but some of them contained a little adipose tissue. The great majority of the cells in the white areas were elongated and ran in bundles or strands (Fig. 18). These cells had elongated nuclei, and the outlines of their cytoplasm were very ill defined. In some situations the cells were much smaller and less elongated; these were regarded as developmentally younger cells but otherwise as of the same nature as those illustrated in Figure 18. Those portions of the tumor which appeared red to the naked eye showed an angiomatous appearance microscopically (Fig. 19). The walls of the vascular channels seemed somewhat hyaline. The elongated cells of the tumor tended to merge insensibly in the walls of the large vessels (Fig. 20). In some situations the dilated vascular channels formed the bulk of the tissue (Fig. 19). The close histologic resemblance that this tumor bore to the renal tumors of case 1 is made clear by comparing Figures 18 and 20 with Figures 2 and 4, respectively. If the arguments in support of a neural origin for the renal tumors of case 1 are valid, then it is thought they give strength to the opinion that neurilemmoblastosis is the essential condition in the renal tumor in case 3.

## "HONEYCOMB LUNG," "CYSTIC DISEASE OF THE LUNG"

The occurrence of cystic disease of the lung and tuberous sclerosis of the brain in the same patient has been recorded by Berg and Vejlens[3] and by Warren and Warvi.[4] It is suggested that the emphysematous lungs in case 1 come under the same category. Pneumothorax occurred in the case recorded by Berg and Vejlens, in the case described by Warren and Warvi, and in cases 1 and 4 of the present series.

It seems important to determine whether cystic disease of the lung, occurring apart from tuberous sclerosis of the brain and its more common accompanying lesions, may present histologic changes like those found in cystic disease of the lung which forms part of the tuberous sclerosis complex in the one individual. Case 4 appears to be an example of cystic disease of the lung occurring apart from tuberous sclerosis of the brain; the brain, however, was not examined at autopsy, and it is

recognized that there may be lesions of tuberous sclerosis in the brain, without clinical evidence of its presence.

CASE 4

The patient (P.U.S. 3326) was a woman, 32 years of age. Her father's brother suffered from "epileptic fits." The patient herself had had no serious illnesses, and had had no previous mental disorders. For 4 years prior to death she had suffered from breathlessness on the least exertion. About 2 or 3 years before death she had "spat up blood." Shortly before death she became cyanosed and dyspneic.

*Autopsy Report*

At autopsy, there was a small right pneumothorax. The left *lung* weighed 400 gm.; the right, 710 gm.; when the lungs were cut no normal pulmonary tissue could be seen; the whole of each lung appeared to consist of small communicating cavities varying from 2 mm. to 1 cm. in diameter, giving a sponge-like appearance to the organ. There was much blood in the right lung. The hilar lymph nodes were not enlarged. The *heart* weighed 300 gm. The right ventricle was somewhat hypertrophied. In the peritoneal cavity were about 5 pints of turbid fluid. The *liver* was small and fatty; the *kidneys* were dark red due to venous congestion. The *brain* was not examined.

Histologic Findings

*Lungs.* Figure 21 shows a cyst-like area immediately beneath the visceral pleura. Figure 22 shows portions of several cystic spaces, some of them partly collapsed; the thick walls of the cystic spaces are composed of spindle-shaped and elongated cells. This cellular tissue did not extend throughout the entire lung; indeed its distribution was patchy and scattered. Occasionally a small nodule of elongated cells was seen. Part of such a nodule is illustrated in Figure 23, and here the elongated cells, with their elongated nuclei and ill defined cytoplasm, run parallel with one another and give an appearance closely resembling that of the renal tumors of cases 1 and 3 (Figs. 2 and 18). The changes in the lungs in case 4 are somewhat different from those in case 1, but in both cases the pulmonary lesions are thought to be essentially neurilemmoblastic.

*Lymph Nodes.* Figure 24 shows the structure of a hilar lymph node from case 4. Lymphoid tissue is seen, but the outstanding feature is presented by the strands of elongated cells, which bear a close resemblance to those in the pulmonary tissue (Figs. 22 and 23). The neurilemmoblastic tissue in the lymph nodes varied somewhat in its structure. In this case another lymph node, certainly hilar or mediastinal, showed in addition to broad bundles of elongated cells like those in Figure

24, clusters of smaller, irregularly rounded or oval cells that are thought to be akin to those in the lymph node in case 1 (Fig. 15).

### *Comment*

The pulmonary changes in case 4 seem to correspond grossly and histologically to those of the case described by Rosendal[5] in which the alveoli and bronchioles were transformed into small cysts, and involuntary muscle was present in the interstitial tissue in the lungs and in the hilar and mediastinal lymph nodes. Also, the pulmonary changes in case 4 seem to correspond grossly and histologically to those of the case described by Berg and Vejlens[3] in which cystic disease of the lung, spontaneous pneumothorax, and tuberous sclerosis of the brain were present in the same patient; these authors said that in the connective tissue forming the walls of the pulmonary cysts there were smooth muscle cells forming groups with tumor-like appearance and a structure resembling that of myomas. It seems likely that the changes in the lungs and lymph nodes in cases 1 and 4 in the present series are essentially of the same nature as those in the lungs and lymph nodes in the case described by Rosendal and in the lungs in the case recorded by Berg and Vejlens, and that it is only the interpretations of histologic appearances that differ.

## Discussion

### *General*

In cases 1 and 2 many of the common extracerebral lesions to be met with in the tuberous sclerosis complex are present. The hypothesis submitted in this paper is that neural intrinsic factor underlies all of them. Neural intrinsic factor is conceived of as having several potentialities. One of these potentialities is in the direction of neurofibromatosis in which the abnormal specific nerve sheath tissue is relatively mature, is closely related to nerve fibers, and is associated with changes of a distinctive kind in various organs and tissues.

A second potentiality is in the direction of neurilemmoblastosis in which the abnormal specific nerve sheath tissue is relatively immature, is unrelated to nerve fibers, and is associated with changes in organs and tissues of a distinctive kind, but different from those met with in association with neurofibromatosis. The induced changes in neurilemmoblastosis and those in neurofibromatosis may overlap, especially in the skin. By virtue of the second potentiality (in the direction of neurilemmoblastosis), neural intrinsic factor may influence the development and behavior of various tissues or organs, and such tissues or organs may show (a) conspicuous evidence of neurilemmoblastosis, (b) slight evi-

dence of neurilemmoblastosis, or (c) no distinctive evidence of neurilemmoblastosis. It is therefore considered that neurilemmoblastosis and its underlying neural intrinsic factor should be regarded as parts of the one concept.

In relation to the preceding case reports there are four suggestions which are regarded as of outstanding importance: (1) That neural tissue, and not involuntary muscle, is the essential component of the cellular tumors in cases 1, 3, and 4; (2) that the fatty tumors in the liver in case 1 are due to the influence of neural tissue on neighboring connective tissue cells; (3) that the kidneys in case 2 show changes characteristic of congenital polycystic disease of the kidneys; (4) that the distinctive lesions of neurilemmoblastosis are dynamic and not static. Cutaneous lesions, for example, usually appear after birth, and often can be observed to increase in size. Static lesions, however, may also occur. The fourth observation means that at least some of the lesions of neurilemmoblastosis cannot be due to extrinsic factors causing sudden injury to, or disease of, a part of the fetus during gestation, but that such lesions may reasonably be accounted for by some intrinsic factor carried in the germ plasm predisposing certain parts of the body to the disease, the actual manifestation of the disease being delayed for even many years after birth. Extrinsic factors theoretically might cause changes in the fetus which would predispose the tissue to lesions appearing later in life, but, at least in the majority of cases, the delayed appearance of such lesions is associated with evidence suggesting germinal transmission of the predisposition to them.

*Points in Favor of Regarding the Cellular Tumors of Cases 1, 3, and 4 as Part of a Systemic Disease (Neurilemmoblastosis) Due to Abnormal Development of Specific Nerve Sheath Tissue (Neurilemma)*

(1). Tuberous sclerosis of the brain is commonly associated with fatty tumors in the kidney like those in case 1. (2). Tuberous sclerosis of the brain is due to distinctive proliferation of developmentally abnormal cerebral glia, and is thus of neural (epiblastic) origin. (3). In the lipomatous tumors of the kidney in case 1 there are cellular portions presenting the same features as the white renal tumors, which are predominantly cellular and contain very little adipose tissue. (4). In case 1 cellular tumors like those in the kidney were present in liver, lungs, uterus, and lymph nodes, so that the condition is widespread and probably of the nature of a systemic disease. (5). The association of the cellular tumors of the kidney in case 1 with adipose tissue is in keeping with a neural affinity of the cellular tissue since lipomata are commonly

associated with neurofibromatosis which is thought to have a neural basis. (6). Parts of the cellular tumors in case 1, especially those in the uterus, are thought to be morphologically different from involuntary muscle. (7). Tumors of involuntary muscle are not commonly associated with overgrowth of adipose tissue. (8). There is a close morphologic resemblance between the elongated-celled tissue of the cellular renal tumors of case 1, the renal tumor of case 3, and certain affected areas in the lungs of case 4, on the one hand, and the tissue of traumatic and spontaneous schwannomas (neurilemmomas), on the other. (9). If the elongated-celled tissue in the lesions of cases 1, 3, and 4 was composed of involuntary muscle (of mesoblastic origin), these lesions would be quite different from the cerebral lesions of tuberous sclerosis which are of epiblastic origin, whereas in the present hypothesis the cerebral and extracerebral lesions are regarded as all of the same order, that is, as parts of a systemic disease of epiblastic origin.

### *Causation and Origin of the Lipomata*

In case 1 the relation of the cellular tissue to the adipose tissue would seem to be the same in the kidneys as in the liver. The interpretation placed on the appearances illustrated in Figures 9 to 12 inclusive is that abnormal cells of neural origin have influenced normal cells of connective tissue origin to store fat, even though such cells never store fat in normal conditions, no matter how obese the subject. This is the more remarkable because a liver may show extensive fatty change of the hepatic parenchyma although the connective tissue cells in the liver framework (*e.g.*, portal tracts) remain quite free from lipids. In some lipomata a hemangiomatous element is present. The overgrowth of adipose tissue in such tumors is probably not due to the extra blood supply, because neural abnormality with little alteration to vessels may be associated with overgrowth of adipose tissue, as is to be seen in certain lesions in both neurilemmoblastosis and neurofibromatosis. It is suggested that in angiomatous lipomata neural intrinsic factor may underlie both the angiomatous element and the lipomatous element of the growth.

### *Pathogenesis of Polycystic Disease of the Kidneys and Associated Conditions*

The renal cysts of case 1 are regarded as probably due to congenital polycystic disease, and the lung changes in case 1 are regarded as essentially of the same nature as those in the cystic lungs associated with tuberous sclerosis described by Berg and Vejlens.[8] The renal cysts in case 2 are regarded as definitely due to congenital polycystic disease, and tuberous sclerosis of the brain and congenital rhabdomyoma (congeni-

tal glycogenic tumor) of the heart were present in the same patient. It would seem that the renal cysts described by Batchelor and Maun[2] as commonly associated with congenital glycogenic tumor of the heart may come into the category of congenital polycystic disease of the kidney. These authors wrote that the majority of congenital glycogenic tumors of the heart are associated with tuberous sclerosis of the brain.

Madonick, Savitsky, and Hochfeld[6] added 2 cases to the 12 already described in the literature of association between intracranial aneurysm and polycystic disease of the kidney. They believed the association is more than coincidental. Their first patient, a man 52 years of age, had a congenital aneurysm of the anterior cerebral artery associated with cysts of the lungs, liver, and kidneys. This is in keeping with the opinion that a relationship exists between congenital polycystic disease of the kidney and faulty development of the circulatory system. The association with cysts of the lungs is in keeping with the suggestion that both congenital polycystic disease of the kidney and developmental abnormalities of vessels at the base of the brain are predisposed to by neural intrinsic factor (or basic intrinsic factor), because evidence is submitted in this paper in support of the opinion that neurilemmoblastosis and its underlying neural intrinsic factor are related to cystic disease of the lung and to developmental abnormalities in the circulatory system. In view of the above statements it is suggested that neural intrinsic factor (or basic intrinsic factor) may underlie congenital polycystic disease of the kidney.

### *The Significance of the Abnormalities of the Circulatory System Associated with Neurilemmoblastosis*

It is thought that in case 1 of the present series the large size of the pulmonary artery, in association with widespread emphysema (honeycomb lung or cystic disease of the lung) and neurilemmoblastosis, was due to abnormal development. In the case of epiloia (tuberous sclerosis of the brain) described by Norman and Taylor,[7] there was a large congenital diverticulum of the left ventricle; in the wall of the diverticulum there was tissue like that of the wall of the aorta (heterotopia of aortic tissue). Madonick, Savitsky, and Hochfeld[6] found a congenital aneurysm in the wall of the anterior cerebral artery associated with cysts of the lungs, liver, and kidneys. It would seem, therefore, that developmental abnormalities of the circulatory system occur in association with neurilemmoblastosis or with some of the lesions which, although they may show no distinctive neurilemmoblastic tissue, are possibly part of the neurilemmoblastic complex; these occurrences are in keep-

ing with the suggestion that neural intrinsic factor (or basic intrinsic factor) may influence the development of abnormalities of the circulatory system on these occasions.

### *Congenital Rhabdomyoma (Congenital Glycogenic Tumor) of the Heart*

Batchelor and Maun[2] stressed the importance of the glycogenic content of the tumor cells. They said that although their review of the literature revealed that tuberous sclerosis had been observed in 50 per cent of cases of congenital glycogenic tumor of the heart, the co-existence of the two lesions actually was much higher. In the present study, congenital rhabdomyoma (congenital glycogenic tumor) of the heart was present only in case 2. This tumor presented the usual histologic features. It is suggested that neural intrinsic factor (or basic intrinsic factor) may have predisposed to the development of this tumor, which is regarded as an anomaly of growth and differentiation. The fact that in Wolbach's[8] case of cardiac rhabdomyoma there was evidence of faulty development of neural tissue as revealed by the presence of nests of neuroglial tissue in the meninges of the spinal cord is thought to be consistent with this opinion. The histologic resemblance between the heart muscle of the normal human fetus at an early stage of development and congenital rhabdomyoma (congenital glycogenic tumor) of the heart is also thought to be in keeping with this interpretation.

Norman and Taylor[7] described a congenital diverticulum of the heart (heterotopia of aortic tissue) in association with epiloia (tuberous sclerosis). Intervening between the diverticulum and the muscular wall of the ventricle was an irregular mass of adipose tissue microscopically showing a very meager stroma. A remarkable feature of this stroma was the presence of scattered cells of large size presenting features which the authors said left little doubt that they are the same as the primitive muscle cells which form the so-called rhabdomyoma of the heart.

In the present paper, evidence has been submitted in support of the opinion that neural intrinsic factor underlies lipomatous overgrowth (Figures 9 to 12), and it has been suggested that neural intrinsic factor (or basic intrinsic factor) may influence the development of congenital aneurysm or dilatation of vessels. Therefore it is thought that the association of heterotopic aortic tissue (in the wall of a congenital cardiac diverticulum) with heterotopic adipose tissue containing primitive cardiac muscle fibers, may be significant in this regard, and may support the suggestion that neural intrinsic factor (or basic intrinsic factor) may influence the development of congenital glycogenic tumor of the heart.

### *Miscellaneous Lesions*

In keeping with the hypothesis submitted in this paper, it is suggested that neural intrinsic factor (or basic intrinsic factor) may influence the development of various other lesions sometimes found in patients suffering from tuberous sclerosis, such as cutaneous fibromata and bony lesions, including melorheostosis as in the case reported by Hall.[9]

## Conclusions

The hypothesis submitted in this paper is that there is a common factor underlying all of the extracerebral lesions comprised in the tuberous sclerosis complex.

It is suggested that this common factor is intrinsic in nature and neural in origin, and to it the name neural intrinsic factor is applied.

Cellular tissue in renal tumors associated with tuberous sclerosis and cellular tissue found in cystic disease of the lung, widely regarded as involuntary muscle, is interpreted as being akin to specific nerve sheath tissue (neurilemma, sheath of Schwann) and as constituting part of a systemic disease for which the name neurilemmoblastosis is used.

Congenital polycystic disease of the kidneys, cystic disease of the lung, and certain developmental abnormalities of the circulatory system are thought to link up with neurilemmoblastosis, possibly at the basic intrinsic factor level.

I wish to thank Drs. A. C. Telfer, R. D. K. Reye, A. H. Tebbutt, and Jean Armytage for providing specimens and notes, Mr. S. Woodward-Smith for taking the photographs and photomicrographs, and Mr. L. Findlayson for technical assistance.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

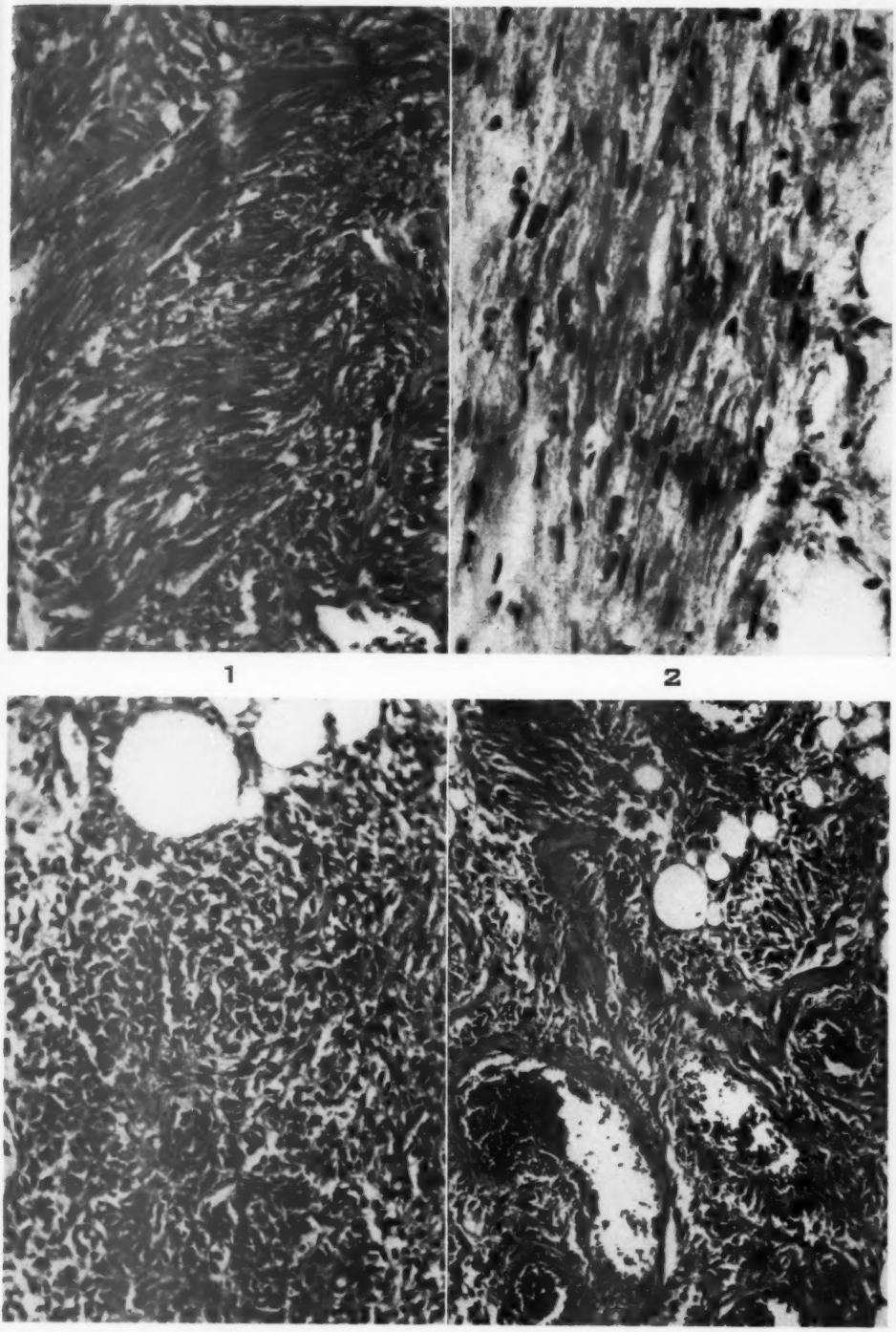
### PLATE 75

FIG. 1. Case 1. Kidney, showing an appearance commonly found in the cellular tumors. × 200.

FIG. 2. Case 1. Kidney: many of the nuclei are long, have parallel sides, and rounded or almost square ends. This figure may be compared with Figure 18, case 3 (kidney), and Figure 23, case 4 (lung). × 400.

FIG. 3. Case 1. Kidney: in this portion of the cellular tissue the individual cells are small, rounded or irregular, and their cytoplasm is ill defined. × 200.

FIG. 4. Case 1. Kidney: small spindle cells are abundant; irregular homogeneous bands are conspicuous. The walls of some blood vessels are composed of homogeneous material; the walls of others show fairly good histologic detail. Dilated vascular channels are conspicuous in this field, but in some fields they are much more prominent and give an angiomatous appearance. This figure may be compared with Figures 19 and 20, case 3 (kidney). A few fat cells are included. × 100.



1 2

3 4

Inglis Neurilemmoblastosis

## PLATE 76

FIG. 5. Case 1. Kidney: a lipomatous tumor is shown; the dark areas in its substance are due to the presence of cellular tissue like that which forms the white tumors. × 4.25.

FIG. 6. Case 1. Kidney: a cyst is seen with epithelial lining (detached in preparing the section), and a thick wall. Appearances like this may be seen in congenital polycystic disease of the kidney. × 50.

FIG. 7. Case 1. Uterus: numerous pale tumor nodules contrast with the dark myometrium. The nodule indicated by the letter A is shown under higher magnification in Figure 8. The letter B points to one of several smaller nodules. × 15.

FIG. 8. Case 1. Uterus: the nodule indicated by the letter A in Figure 7 is here shown more highly magnified. A small portion of the myometrium (dark) is included in this illustration. The tumor (pale) resembles tumors in other organs; for comparison with Figure 1 (kidney). × 100.

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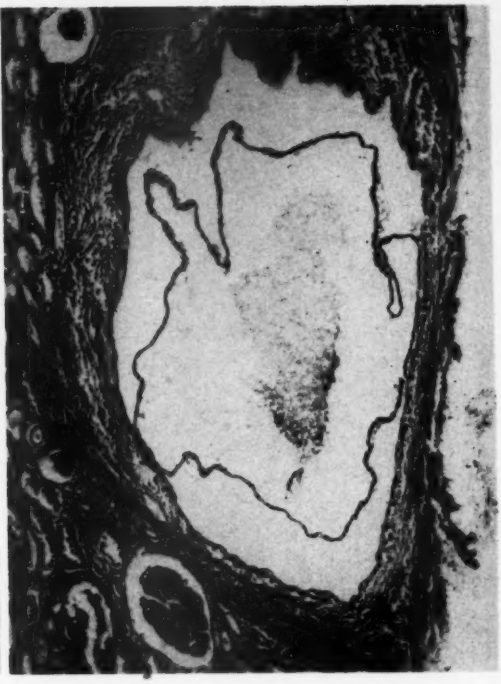

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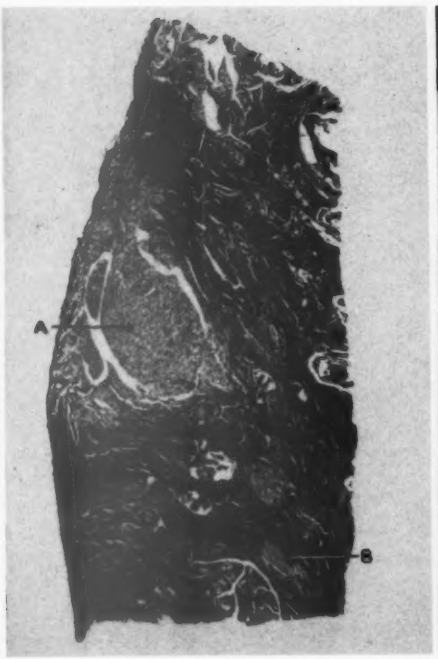


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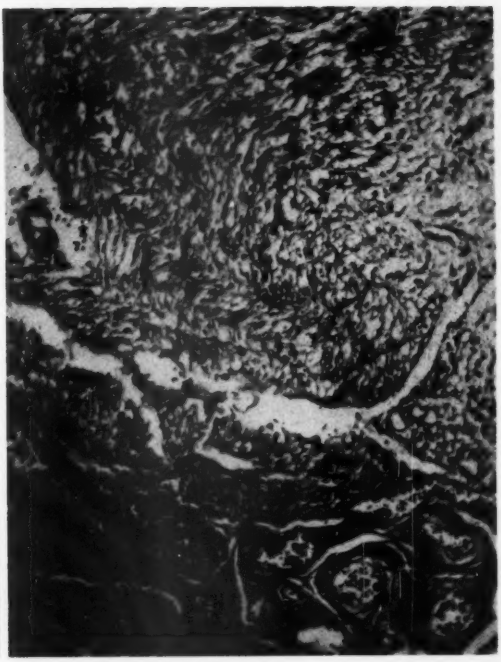

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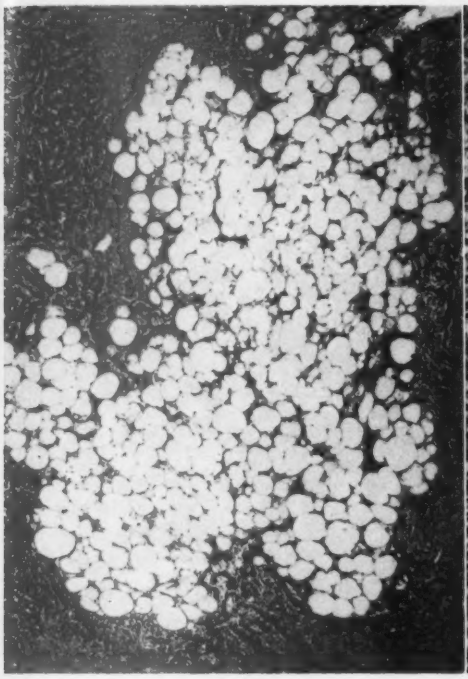
## PLATE 77

FIG. 9. Case 1. Liver: lipomatous tissue is present in congested hepatic parenchyma. The lipoma is ill defined at the periphery, and isolated fat cells are present nearby. × 60.

FIG. 10. Case 1. Liver: abnormal tissue is seen in a portal tract. A bile duct (A) is present below and a part of a branch of the portal vein above. Most of the tissue is composed of spindle cells and structureless material. Fat cells are distributed irregularly throughout this abnormal tissue which is thought to be of the same nature as the tumors in the kidneys and elsewhere. × 100.

FIG. 11. Case 1. Liver: a portal tract is seen surrounded by a zone of relatively normal hepatic parenchyma; the more distant parenchyma shows retrograde change due to venous engorgement. In the center of the portal tract is a dilated branch of the portal vein; bile ducts are small and barely recognizable. The specially significant features are the ill defined, loosely arranged tissue and the fat cells. It is thought that the changes in this portal tract are essentially the same as those in the portal tract illustrated in Figure 10. × 100.

FIG. 12. Case 1. Liver: the cellular lesion in the central part of this illustration is regarded as a tumor nodule like those found in other organs. This figure may be compared with Figure 13 (lung) and Figure 15 (lymph node). × 200.



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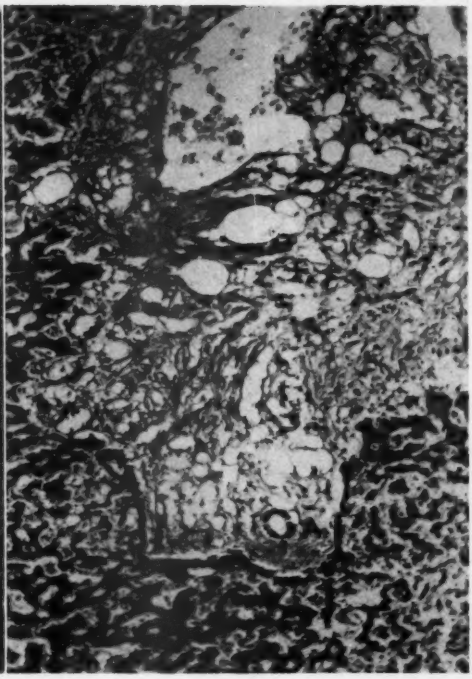

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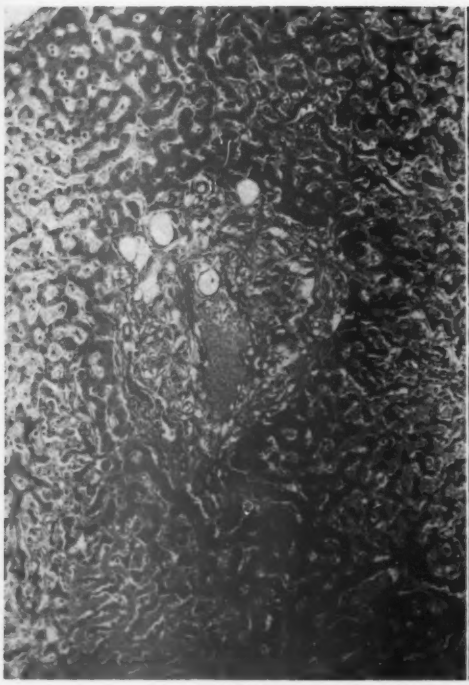

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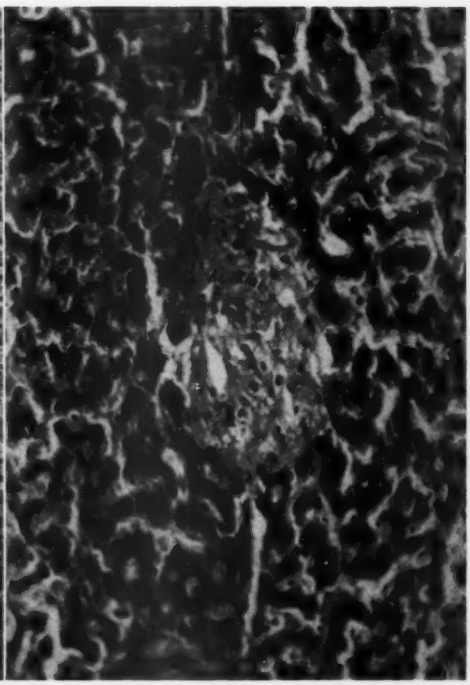

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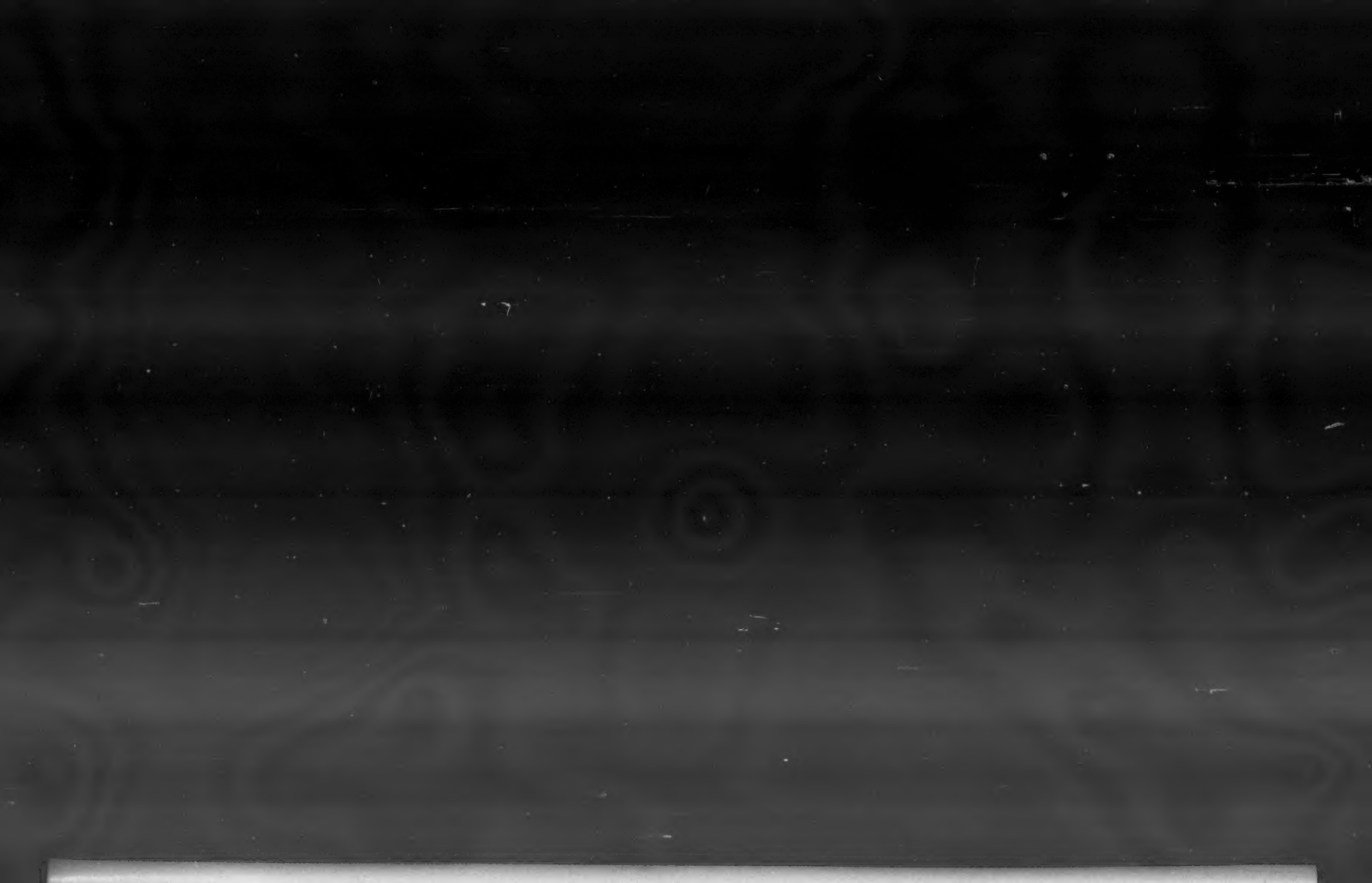
PLATE 78

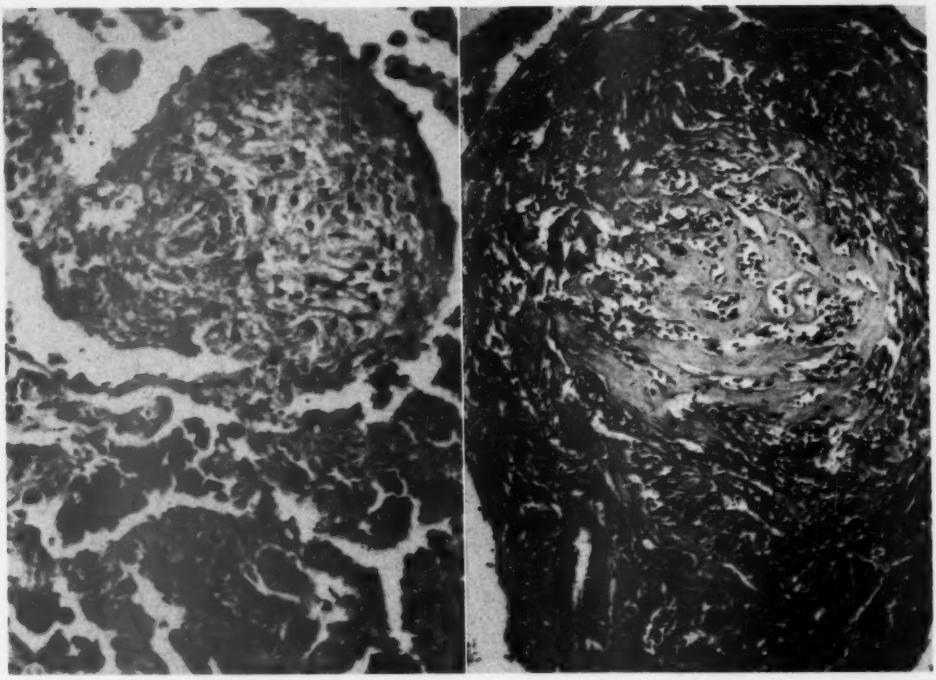
FIG. 13. Case 1. Lung: the lower part of the illustration shows somewhat collapsed air sacs, the lumina of which contain cells laden with hemosiderin; the upper part shows a tumor nodule in which the cells are ill defined. This figure may be compared with Figure 12 (liver) and Figure 15 (lymph node). × 200.

FIG. 14. Case 1. Lung: a tumor nodule is seen in which irregular homogeneous bands are conspicuous, for comparison with Figure 4 (kidney). × 100.

FIG. 15. Case 1. Lymph node: a small tumor nodule is present. This figure may be compared with Figure 12 (liver) and Figure 13 (lung). × 200.

FIG. 16. Case 2. Kidney: cysts are present into which glomerular tufts project. × 100.





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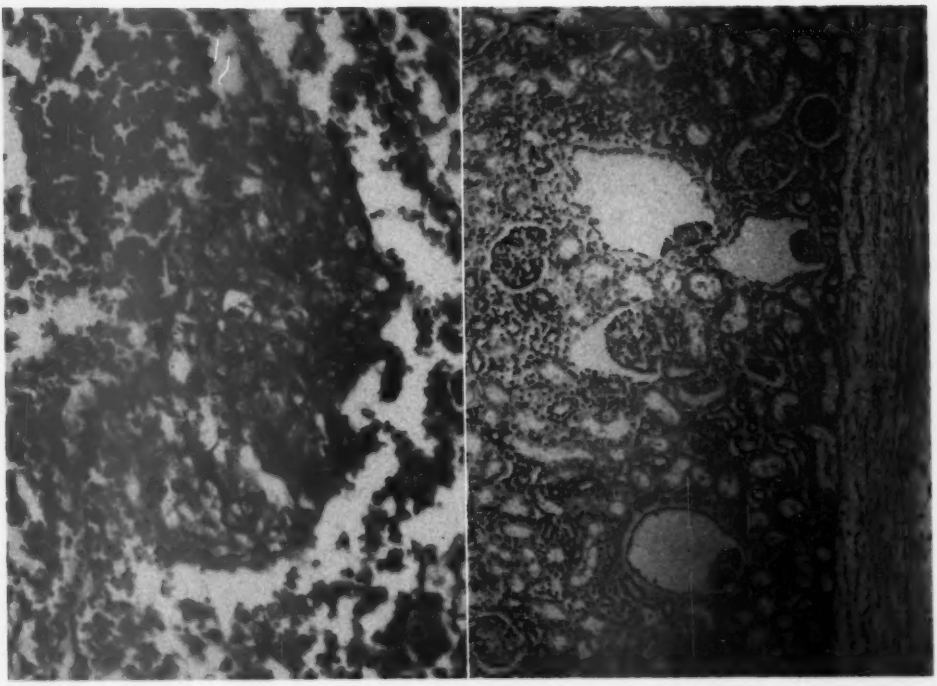

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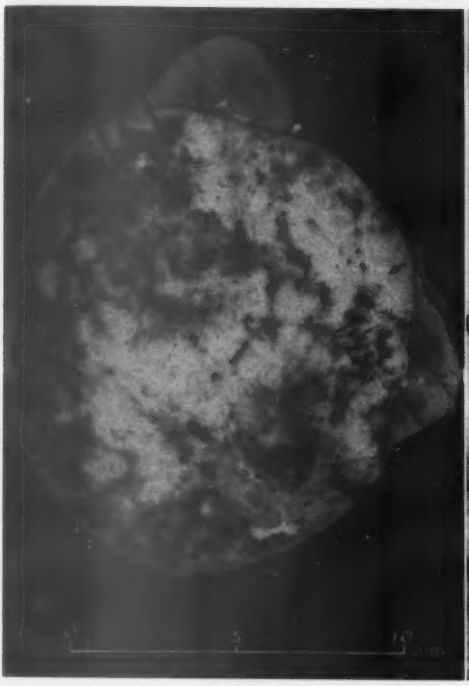
PLATE 79

FIG. 17. Case 3. Kidney: the photograph shows the gross appearance and size of the tumor.

FIG. 18. Case 3. Kidney: adipose tissue is included, but the most striking feature is presented by the elongated cells which have ill defined outlines and conspicuous nuclei with parallel sides and rounded ends; this may be compared with Figure 2, case 1 (kidney), and Figure 23, case 4 (lung). × 200.

FIG. 19. Case 3. Kidney: this portion of the tumor has an angiomatous appearance. × 50.

FIG. 20. Case 3. Kidney: the tumor cells are seen to merge in the walls of the vascular channels. Some lipomatous tissue is included. × 100.



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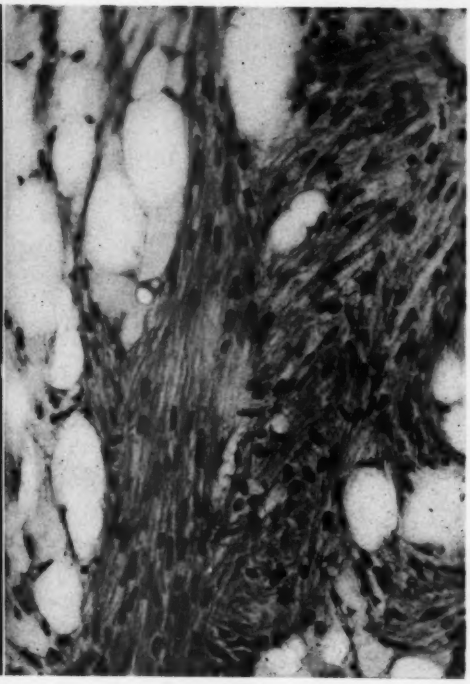

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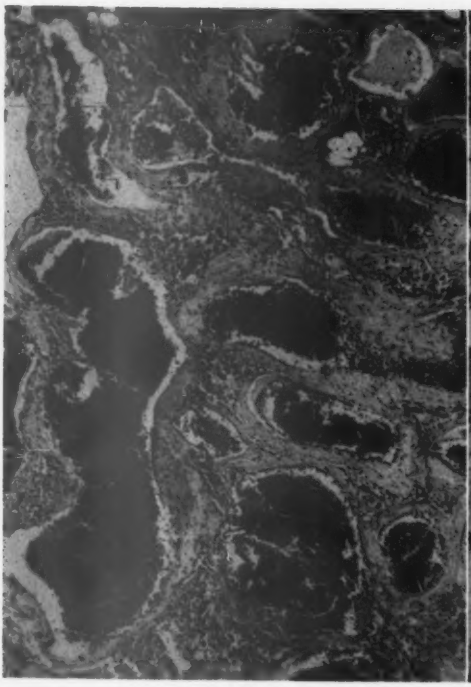

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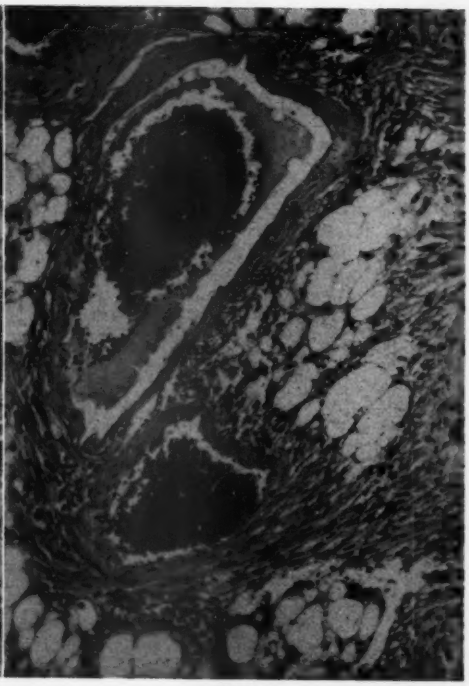

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Inglis Neurilemmoblastosis

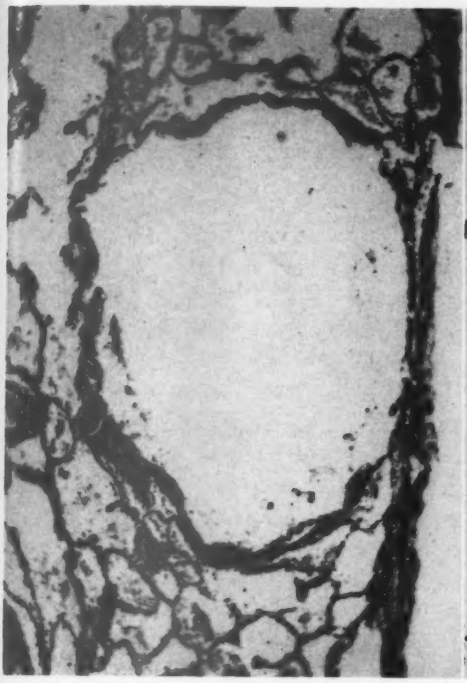
PLATE 80

FIG. 21. Case 4. Lung: a cyst-like area is seen immediately beneath the visceral pleura. × 50.

FIG. 22. Case 4. Lung: the walls of partly collapsed cyst-like spaces are composed largely of spindle-shaped and elongated cells. × 200.

FIG. 23. Case 4. Lung: this is a portion of a small nodule composed largely of elongated cells with elongated nuclei having parallel sides and rounded ends; this may be compared with Figure 2, case 1 (kidney), and Figure 18, case 3 (kidney). × 400.

FIG. 24. Case 4. Lymph node: the illustration shows the structure of a portion of a hilar lymph node; bundles of elongated cells are conspicuous. × 100.



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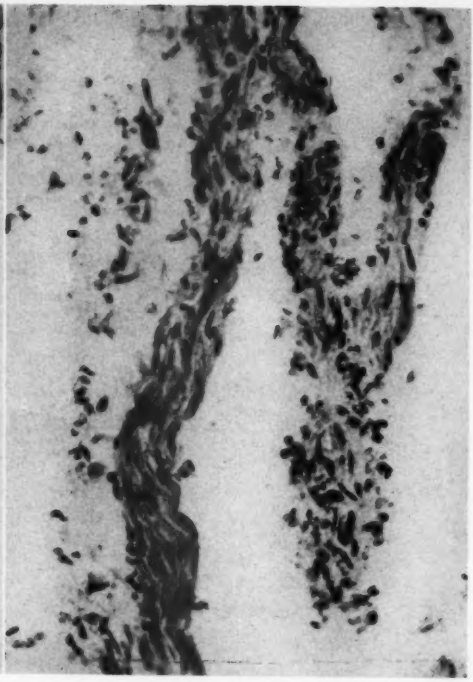

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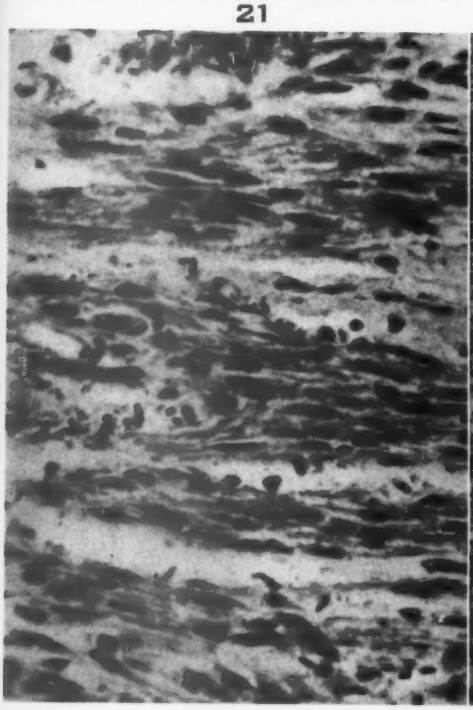

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Inglis

Neurilemmoblastosis

# THE PATHOGENESIS OF EXPERIMENTAL FAT EMBOLISM *

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The literature concerning fat embolism is abundant and has recently been surveyed comprehensively in the publications of Wilson,[1] Warren,[2] and Robb-Smith.[3] It is noteworthy, however, that among the various studies, whether clinical or experimental, the stress has been placed on the action of the fat upon the host. The condition of the patient and of the experimental animal has received incidental attention; it has been tacitly assumed that the host is biologically invariable and uniformly responsive, regardless of the previous history and treatment. That the response is remarkably variable is indicated by the results attained through the thorough search of routine autopsies for evidence of fat embolism. Such studies[4,5] reveal a very high incidence of fat embolism among cases of traumatic injury, especially with fractures, although the mortality due directly to fat embolism is low and unpredictable. Robb-Smith has reported similar findings and attributes much importance to the fat as a lethal factor. In view of this, the present study was planned to elucidate the behavior of the host toward fat embolism and to clarify the factors which might affect the response either favorably or adversely.

## METHOD

Fat embolism was produced in albino rabbits, weighing 2,000 to 3,000 gm., of both sexes, by the injection of homologous liquid fat into the right ear vein. This fat was obtained from the perirenal fat pads of normal rabbits. It was finely minced, mixed with one and one-half volumes of 95 per cent ethanol, and homogenized in a Waring blender for 2 minutes. This homogenate was refluxed at 80° to 90° C. for 12 hours and subsequently filtered through a warm Buchner funnel to eliminate coarse, insoluble residue. The filtrate was allowed to stand in a separatory funnel at 38° C. for an additional 12 hours in order to allow separation of fat crystals insoluble at this temperature. Final exclusion of the insoluble fraction was achieved by rapid centrifugation. The extract was a clear, straw-colored liquid of low viscosity when the residual alcohol had evaporated.

Injections of this homologous fat were made into the ear vein at a

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rate approximating 0.4 cc. per minute, so calculated to permit formation of small, separate fat globules in the vein. The droplets could be visualized by transmitted light. After injections, the animals were returned to their cages and observed for respiratory, cerebral, and cardiac abnormalities. The animals that died were autopsied, the organs examined for gross alterations, and ample representative portions fixed in a 4 per cent solution of formaldehyde. Some of the surviving animals were sacrificed and similarly autopsied. The tissues were subsequently embedded in paraffin, sectioned at 8 $\mu$, and stained with hematoxylin and eosin. From selected samples of the formalin-fixed lung and liver, frozen sections were taken and stained with sudan III.

## Experimental Procedure

### *Effect of Dosage, Rate of Injection, and Repeated Injection*

The amount of fat, injected at constant rate, was varied to include levels of 0.45, 0.55, 0.75, and 0.90 cc. of fat per kg. of body weight. In rabbits receiving doses of 0.90 cc. per kg., death occurred within 10 to 30 minutes in all animals, whereas in those receiving 0.75 cc. per kg., death was similarly rapid although the mortality was less. With the smaller doses the animals survived for at least 5 hours and often up to 24 hours. Such animals as survived over 24 hours recovered completely, regardless of the severity of the initial pulmonary signs, and in no instance was a late death encountered for as long as 120 days after injection. The mortality rate (Table I) varied directly with the dosage, 0.55

Table I

*Effect of Dosage upon Mortality by Fat Embolism* *

| No. of animals | Dosage | Survivors | Fatalities | Mortality |
|---|---|---|---|---|
| | *cc./kg.* | | | % |
| 18 | 0.45 | 15 | 3 | 16.6 |
| 12 | 0.55 | 6 | 6 | 50.0 |
| 6 | 0.75 | 2 | 4 | 66.0 |
| 6 | 0.90 | 0 | 6 | 100.0 |

* The rate of injection was constant for all animals.
Injections were made into the right ear vein, without anesthesia.

cc. per kg. giving a 50 per cent lethal result. Since the rate of injection was constant, it was excluded as a factor in varying mortality. As a further test of rate, a series of rabbits were injected with fat as rapidly as technically feasible without altering the mortality rate above the previous level.

In a group of animals which survived the infusion of 0.45 cc. per kg., repeated injections were administered at staggered intervals of 110 hours, distributed for a total of four injections. On the basis of this,

it was expected that adaptation to the fat might reveal itself by a lowered mortality with succeeding injections. It was found under the circumstances that the mortality rises slightly with the second and third fat injections until 75 per cent of the animals succumbed. No evidence of tolerance to repetitive fat embolism was observed.

### *Effect of Tourniquet Shock and Dehydration*

Tourniquets were applied to the hind legs of rabbits as described elsewhere.[6] Subsequent to a period of 4 hours of complete ischemia, the tourniquet was released. Three hours after release, when swelling of the limb usually is maximal, 0.45 cc. of fat per kg. were injected. It is apparent (Table II) that the mortality rate is considerably elevated in the animals with tourniquet shock when compared with control groups which received only fat injection or which were subjected to ischemia alone. Although the mortality was thus increased, the time of survival was not altered appreciably; no death occurred before 5 or after 24 hours subsequent to injection.

In view of the considerable quantity of fluid which accumulated and pooled extravascularly in the ischemic limb, the effect of dehydration was studied in another group. The animals were allowed dried food but deprived of water for 72 hours. At the 48th hour each was injected intraperitoneally with 30 cc. of 25 per cent glucose to facilitate the dehydration. Fat was administered intravenously in a dose of 0.45 cc. per kg. at the end of 72 hours. In this series 50 per cent of the animals

TABLE II

*Effect of Tourniquet Shock, Dehydration, and Oxygen upon Mortality Rate*

| No. of animals | Fat | Shock | Dehydration | $O_2$, 80% | Survivors | Fatalities | Mortality |
|---|---|---|---|---|---|---|---|
| | cc./kg. | | | | | | % |
| 18 | 0.45 | − | − | − | 15 | 3 | 16.6 |
| 12* | 0.45 | + | − | − | 7 | 5 | 41.6 |
| 18 | − | + | − | − | 18 | 0 | 0.0 |
| 18† | 0.45 | − | + | − | 9 | 9 | 50.0 |
| 12 | 0.55 | − | − | − | 6 | 6 | 50.0 |
| 12‡ | 0.55 | − | − | + | 12 | 0 | 0.0 |

* Three hours elapsed between the release of the tourniquet and the injection of fat.
† All of these animals were deprived of fluid for 3 days before injection of the fat.
‡ Oxygen percentage of effluent air was tested by the Beckman oximeter.

died following the injection (Table II) and in the majority of instances succumbed within 30 minutes in a manner similar to those injected with greater quantities of fat. Neither dehydration nor fat alone produced such a high mortality.

In a series of dehydrated animals, both loss of weight and alterations in hematocrit levels were followed. Hematocrit levels were estimated

by the capillary method.[7] The hematocrit readings and weight changes were taken at the start, and at the 48th and 72nd hour of dehydration. Variation was computed as a percentage alteration of the initial readings. From these studies it has become manifest that neither the magnitude nor direction of change in the hematocrit suggested the outcome of any particular experiment. The weight loss, presumably due to fluid depletion, similarly gave no indication of how the particular animal would respond. Loss of weight varied between 10 and 18 per cent of the original body weight. Dehydration, like tourniquet shock, altered the mortality in a striking manner, whether or not evidence of hemoconcentration was manifest. The mortality rate was identical in both sexes.

### *Effect of Oxygen upon Mortality*

A group of animals which had received 0.55 cc. per kg. of fat were put into a sealed metal chamber with glass ports, immediately at the end of the injection. Oxygen was passed through the chamber under positive pressure from a pressure cylinder at a rate sufficient to maintain the percentage of the effluent gas at between 80 and 82 per cent. Hourly tests were made with a Beckman oximeter over a period of 30 hours. Both food and water were allowed *ad libitum*. Although these animals experienced marked tachypnea and in some instances severe dyspnea, within the period of the experiment none died. At the 30th hour all were killed and autopsied. The administration of oxygen in high volume altered the mortality rate from the 50 per cent seen in the controls to nil (Table II). The duration of 30 hours was set because among the controls fatalities occurred before the 24th hour. The control animals which survived over 24 hours were killed also to permit collateral estimation of visceral changes in the absence of oxygen among survivors.

### Autopsy Findings

The only lesions observed in animals which died rapidly were scattered petechiae in the serosa and slight pulmonary congestion. In the remainder which survived for longer than 5 hours or were subsequently killed, extensive changes were observed in the viscera. The difference between the groups as related to period of survival is demonstrated in Table III.

*Lungs.* In all animals which survived longer than 5 hours the lungs were heavy, and contained gross areas of hemorrhage and scattered small areas of atelectasis. Congestion was extensive. In a few animals pleural effusions measuring 5 to 6 cc. were found. The weight of the lungs was greatly increased from the normal average of about 14.5 gm.

to an average of 35.1 gm., due mainly to congestion and edema, for the weights were only slightly increased, if at all, in those dying immediately after injection when congestion and edema were slight. In the animals receiving oxygen therapy the increase in weight of the lung was less conspicuous and averaged 25.5 gm.

TABLE III

*Duration of Survival of Animals Dying of Fat Embolism*

| No. of animals | Dosage | Agent | Duration | Pulmonary lesions |
|---|---|---|---|---|
| | *cc./kg.* | | | |
| 18 | 0.45 | — | 5–24 hours | + |
| 12 | 0.55 | — | 5–24 hours | + |
| 6 | 0.75 | — | 15–60 minutes | — |
| 6 | 0.90 | — | 10–30 minutes | — |
| 18 | 0.45 | Dehydration | 10–60 minutes* | Slight |
| 12 | 0.45 | Tourniquet | 5–10 hours | + |
| 12 | 0.55 | Oxygen | At least 30 hours | + |

* One animal lived for 7 hours, another for 24 hours.

Microscopic studies of the lungs revealed extensive hyperemia (Fig. 1), extensive pulmonary edema, and in most instances a moderate interstitial infiltration of polymorphonuclear leukocytes.* As previously indicated, the extent of these changes was dependent first upon the duration of survival (Table IV). Among both survivors and non-sur-

TABLE IV

*Lesions of Various Viscera in Relation to Duration of Survival after Fat Injection*

| Lesions | 10–60 minutes | 5–24 hours | Sacrificed at 30 hours | 80% oxygen | Dehydrated* |
|---|---|---|---|---|---|
| Pulmonary edema | | | | | |
| 0 | 10 | 0 | 0 | 2 | 4 |
| + | 4 | 5 | 5 | 5 | 11 |
| ++ | 0 | 3 | 1 | 4 | 2 |
| +++ | 0 | 7 | 3 | 1 | 0 |
| Total | 14 | 15 | 9 | 12 | 17 |
| Hepatic necrosis | 0 | 7 | 5 | 0 | 2 |
| Myocardial necrosis | 1 | 6 | 3 | 6 | 2 |

* Except for 2 animals, all died within 1 hour after injection of fat.

vivors severe grades of involvement were found in about the same proportion. Oxygen therapy, on the other hand, ameliorated the degree of edema and congestion considerably, although alterations were absent in only a few animals. All lungs contained a large number of fat globules, which lodged in both the arteries and capillaries.

* In a recent autopsied case of human pulmonary fat embolism, a similar interstitial leukocytic reaction was seen.

An interesting alteration occurred in the medium-sized arteries. These vessels (Fig. 2) were loose meshed, with separation of the medial cells from each other. In the subintimal connective tissue there was a marked vacuolization and ballooning. The effect of the alterations was a constriction of the caliber of the vessels by the edema of the walls. This ballooning of the intima was not due to imbibition of fat, since by frozen section none was demonstrable within the wall. In most instances the perivascular lymphatics were conspicuously filled with an eosinophilic fluid coagulum. These arterial lesions were absent or minimal in the animals dying rapidly and in those treated by oxygen.

*Heart.* Numerous petechial hemorrhages were scattered over the pericardium and seen frequently beneath the endocardium of the left ventricle. The right ventricle was in most instances dilated and contained grossly perceptible fat globules. Microscopically, there was extensive congestion and stasis of the myocardium. Many focal areas of necrosis (Fig. 3) were observed in which the muscle fibers had undergone lysis, and there were accumulated both monocytes and neutrophilic polymorphonuclear leukocytes. These necrotic foci were predominantly in the right ventricle, although also present elsewhere. They were rare in animals dying suddenly and most numerous in those which survived for some period of time. Oxygen did not preclude their formation or restrict their extent.

*Liver.* Grossly, the liver was intensely congested and enlarged. This was reflected in the usual microscopic picture of congested sinusoids and central veins. Parenchymatous degeneration was frequent, and in a few animals fatty metamorphosis was conspicuous. As is indicated in Table IV, the animals which survived over 5 hours or were sacrificed at 24 to 30 hours frequently suffered an extensive centrilobular necrosis, which destroyed up to three-fourths of the parenchyma in some instances (Fig. 4). There was concomitant leukocytic infiltration and some peripheral parenchymatous degeneration. The frequency of this type of necrosis was 25 per cent and appeared consistently in animals with more severe degrees of pulmonary involvement. Direct toxic action of the fat upon the liver was considered, but it was found that the necrosis occurred also after injection of paraffin oil, which is chemically bland. On the other hand, the animals treated with oxygen never developed necrosis; the livers of these animals were very congested, although the liver cords were normal.

*Kidneys.* There were no gross lesions in the kidneys. Microscopically, many glomeruli contained intracapillary fat globules; and as many as

40 to 60 per cent of the glomeruli might contain them. Signs of necrosis and inflammation were absent, even in glomeruli packed with fat. Fat was observed also within the lumina of the various segments of the nephron, particularly in animals which lived for some time. It appeared bland because, even though large globules filled cells occasionally, no evidence of necrosis was found throughout the nephron. It is pertinent to indicate the difference in the response of the renal and pulmonary tissues to the fat emboli: the former were not reactive, whereas the latter underwent profound inflammatory alterations.

*Other Tissues.* Among the other organs the brain was most closely studied. No gross lesions were detected in any instance. In a rare animal a few scattered petechiae were observed in the cerebellum without any concomitant perivascular necrosis. Lesions were consistently absent in the spleen, adrenals, ovaries, testes, skin, intestines, and aorta. In almost all animals, except those treated with oxygen, numerous petechiae were found in the thymus. A few animals suffered either pleural effusion or ascites.

## Discussion

The mechanisms by which fat embolism is initiated are several and include such diverse conditions as fractures of long bones, orthopedic manipulations, crushing injuries, concussion, injection of oily fluids, infusion of ether, and the hyperlipemia of diabetes mellitus. Whatever the mode of origin, the crux of the problem lies in the method of disposal of the particulate fat. Fat particles below 8 $\mu$ in diameter are dealt with effectively by the organism, whereas those of larger size may behave as emboli. The response of an animal to the infusion of particulate fat into the circulation, regardless of route of entry, will depend considerably upon its ability to cope with the embolic manifestations. This presumes that the fat is chemically inert, and the present studies upon the relationship of dosage and mortality support the view that the animal initially reacts to the fat particles as to any inert, small emboli. Should the animal survive the initial insult, which depends mainly upon the quantity of fat, its subsequent behavior will be conditioned not only by action of the fat upon the tissues but also by the physiologic adaptability of the particular animal and its various viscera to the fat particles.

In the group of animals dying immediately after the fat infusion, death may be ascribed to massive plugging of the pulmonary vessels. Since no morphologic alterations were found in the lungs, chemical action by the fat may be excluded. Among animals which survived the injection by several hours, several viscera may manifest profound alterations.

It is requisite to determine whether the visceral lesions are due to direct action of the fat upon the tissues or are caused by systemic disturbance due to occlusive vascular obstruction by the fat. It might be presumed by some that, if the fat acts chemically, it will exert its effect indiscriminately in whatever tissue it comes to lodge. This is not so. For, although numerous fat emboli are caught up in the kidney and a few in the brain, these organs are singularly free of inflammatory reactions such as are common in the lungs. Furthermore, necrosis of the liver is encountered in the absence of demonstrable fat in that organ. Accordingly, it may be assumed that reaction of the tissues, and therefore of the animal, is decided by factors independent of the nature of the fat itself.

The first problem involves, therefore, the cause of the different reactions by the various organs to the fat. Although the quantity of fat contained in the pulmonary vessels exceeds that in any other organ, it might be expected that the type of reaction, if purely chemical and nonspecific, would vary quantitatively and not qualitatively. But the striking absence of renal lesions, although fat may be concentrated considerably in the kidneys, indicates that the tissues react qualitatively and individually. The absence of edema and inflammation in the lungs of animals injected with paraffin excludes anoxia and ischemia, and suggests that in the lungs the tissues may so act upon the fat as to release irritant substances, whereas such is not the case in other tissues. Lipase has been demonstrated histochemically[8] in the alveolar septa of the rabbit lung and may implement the inflammation by release of fatty acids which are known to cause severe pulmonary inflammation in minute quantity.[9] Furthermore, if the pulmonary lesions were due to anoxia or ischemia, it might be anticipated that they would be obviated by oxygen therapy. Yet, although oxygen ameliorates the changes, it does not exclude them.

On the other hand, the effect of oxygen in abolishing mortality supports the belief that the consequences of the pulmonary lesions, either anoxia or asphyxia, are directly responsible for death of the animals. The absence of hepatic necrosis in the oxygen-treated animal is consistent with this interpretation. Even with persistent severe pulmonary edema, oxygen prevents the occurrence of hepatic necrosis, affording proof that the hepatic lesions are due to anoxia, although those in the lungs are not. Moreover, it is interesting that injections of paraffin oil may cause hepatic necrosis, although the only pulmonary involvement is the plugging of the small vessels of the lung. The cardiac lesions are not affected by oxygen therapy and are, therefore, presumed to be due to emboli, which cause lesions known as Buchner's hypoxemic myo-

cardial necrosis.[10] The efficacy of oxygen therapy in experimental fat embolism indicates its specificity in this condition. It also emphasizes the few reports of dramatic results obtained clinically by the use of adequate, sustained oxygen therapy. Kolmert[11] was the first to discover the remarkable and, as he stated, surprising value of oxygen. Robb-Smith[3] and, more recently, Dunphy and Ilfeld[12] have substantiated the therapeutic value of persistent high oxygen concentration.

Thus it is apparent that the tissues react to the fat differentially and that the local effects may be separated from the systemic. It is clear also, from the behavior of animals either in tourniquet shock or dehydration, that the previous experience of the animal influences in considerable measure the response to fat. The mortality rate is significantly enhanced and the manner of death altered. From an acute variety of death the mechanism is converted to a peracute type by dehydration. Although the effect is striking, it cannot be correlated with any consistent change in either weight loss or hematocrit values. It may be presumed that when the hematocrit reading is elevated the extravascular fluid is depleted. Nevertheless, some animals with marked hemoconcentration and extravascular dehydration survive, whereas others with little or no detectable change succumb to the fat injection. This lack of correlation between manifest dehydration and fatality, and, conversely, the occurrence of fatality without hemoconcentration, are indications that the lethality of water deprivation may be operative in a manner more subtle than depletion of the fluid reserves alone. Whatever the underlying mechanism, it is indisputable that either rapid dehydration or tourniquet shock disadvantageously changes the animal's behavior toward fat embolism.

## Summary and Conclusions

Injection of homologous fat into rabbits causes a mortality and mode of death correlated closely with the dosage given.

Mortality is increased by either dehydration or tourniquet shock.

Death is prevented by oxygen therapy, which also prevents hepatic necrosis.

Death by fat embolism is determined not only by the quantity and quality of fat entering the circulation, but it is influenced by the preconditioning of the animal, which alters its response to the injected fat.

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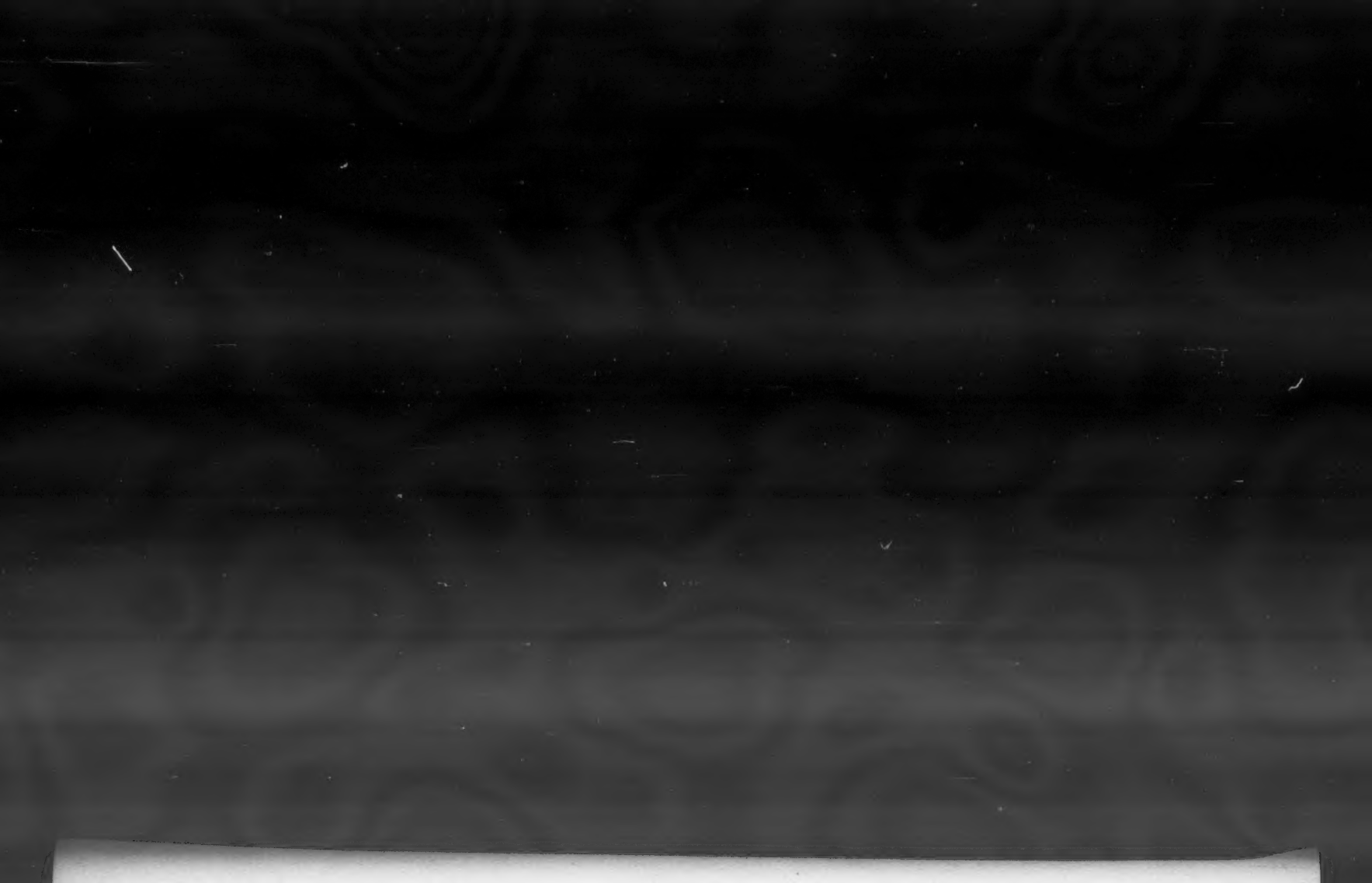
## DESCRIPTION OF PLATES

### PLATE 81

FIG. 1. Section of lung from a rabbit which was injected with 0.55 cc. of fat per kg. of body weight. The animal died 12 hours after injection. There are many capillaries filled with fat vacuoles and the alveoli are filled with coagulated edema fluid. The alveolar septa are congested and contain many interstitial polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 175.

FIG. 2. In the pulmonary artery of the same animal from which Figure 1 was made, there is extensive vacuolation of the subintimal tissue, which lifts off the lining endothelium of the artery. The muscle cells of the media are conspicuously separated by interstitial fluid. Hematoxylin and eosin stain. × 350.



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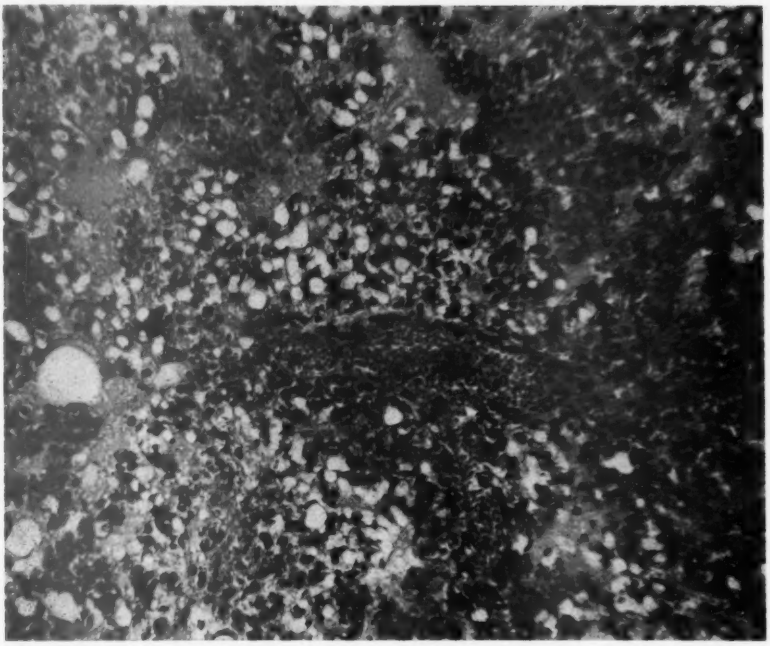

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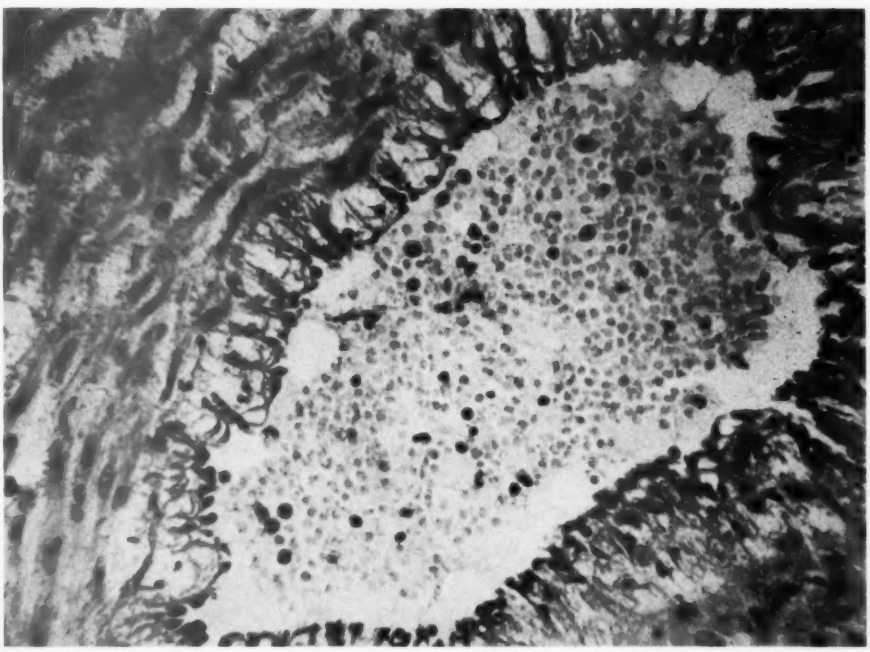

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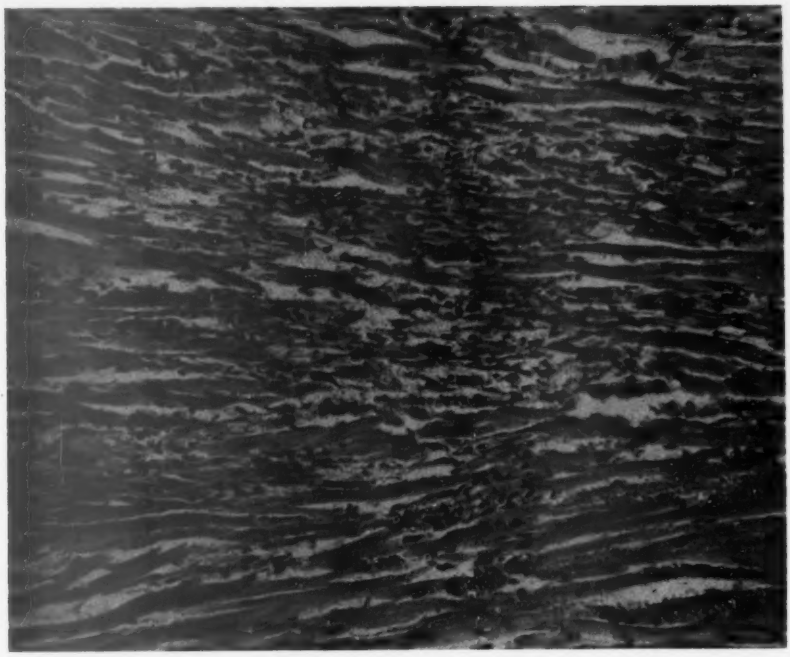
PLATE 82

FIG. 3. The cardiac muscle fibers are necrotic, and there is infiltration of polymorphonuclear leukocytes and monocytes. Many muscle fibers have undergone lysis and granular degeneration. This animal was injected with 0.55 cc. of fat per kg. of body weight. Hematoxylin and eosin stain. × 350.

FIG. 4. The liver of an animal which died 12 hours after injection of 0.55 cc. of fat per kg. of body weight shows extensive centrilobular necrosis with many infiltrated polymorphonuclear leukocytes. Hematoxylin and eosin stain. × 65.

3



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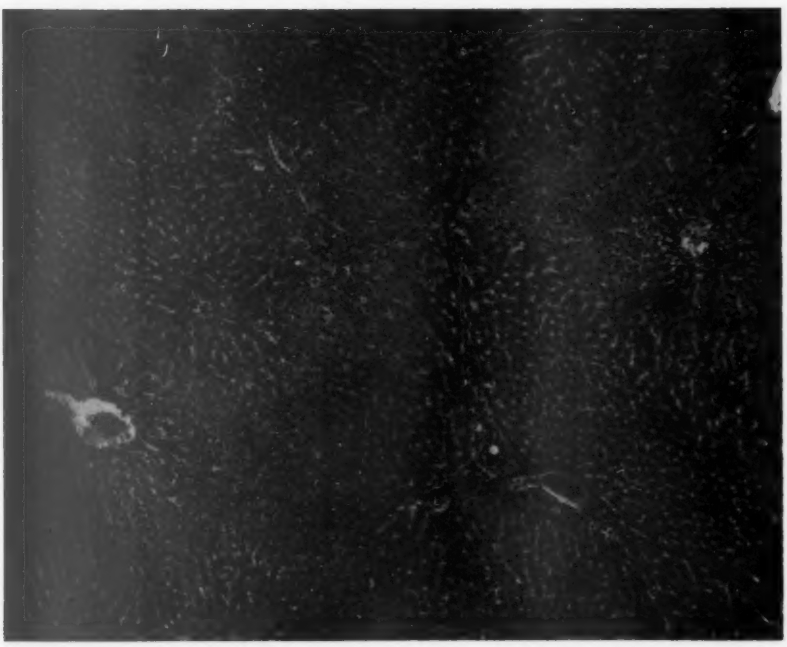

Harman and Ragaz Experimental Fat Embolism

# TERATOMA OF THE NECK IN THE REGION OF THE THYROID GLAND
## A Review of the Literature and Report of Four Cases *

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Teratoma of the neck in the region of the thyroid gland is a rare and usually benign neoplasm. A number of tumors of this region were reported during the seventeenth, eighteenth, and first one-half of the nineteenth centuries. Undoubtedly, many of these were teratomata. However, because of lack of microscopic description, it is believed that these cases should not be accepted and they will not be discussed here. Abstracts and complete bibliographies may be found in the dissertations of the early 1900's, especially that of Pellegrini in 1913, and in the more recent papers of Saphir in 1929 and Pusch and Nelson in 1935.

The first proved case of teratoma of the neck was published by Hess in 1854, and was restudied microscopically by Wetzel in 1895. Since the time of Hess, 60 cases have been added to the literature. The individual cases are listed in the two accompanying tables. Table I includes the tumors reviewed by Saphir, and Table II contains all other probable teratomata of the neck in the region of the thyroid gland. (Style of tables after Saphir.)

Five probable teratomata of this region are placed in Table II, and in the bibliography, upon rather scanty evidence of their true nature, for neither the originals nor abstracts were available. The instance of Munker in 1898 is classed here because of its suggestive title. Hagenbach-Burckhardt's case in 1899 is proposed because of its title, and also because it was found in v. Khautz's report in 1910 of teratomata of this nature. Weyl's case in 1900 is also in the list because Ewing mentions it as a teratoma in his "Neoplastic Diseases." The reports of Magnin in 1942 and Marescot Iglesias in 1945 are included because of their titles. It is hoped that the incorporation of these 5 cases may be of some value to future investigators. Eliminating the above 5 cases leaves a total of 56 tumors collected from the literature which will be discussed in this communication.

Early extensive accounts were presented by Poult in 1905, Hunziker in 1909, Pellegrini in 1913, and Ehlers in 1914. Ehlers pointed out that the first case described by Flesch and Winternitz in 1905 was not a teratoid tumor. Subsequent restudy proved the correctness of Ehler's statement. More recent extensive compilations are those of Saphir in

* Received for publication, June 27, 1949.

TABLE I

*Additional Information About the Tumors Reviewed by Saphir in 1929. The Statements of the Various Authors Concerning the Presence of Thyroid Tissue in the Tumor, and Concerning the Relationship of the Tumor to the Thyroid Gland, Are Presented*

| No. | Author | Year | Thyroid tissue present in tumor | Displacement of thyroid gland | Hydramnios present at birth | Operation performed | Thyroid arteries entering tumor |
|---|---|---|---|---|---|---|---|
| 1 | Hess | 1854 | Thyroid gland separated by fibrous capsule from tumor | Displaced right lobe | No ref. | No | No ref. |
| 2 | Zahn | 1886 | Described "follicles" of embryonic thyroid tissue in tumor | Displaced | No | No | No ref. |
| 3 | Schimmelbusch | 1894 | None | Yes | No | Yes | No ref. |
| 4 | Wetzel | 1895 | Same as case 1 | Displaced right lobe | No | No | No ref. |
| 5 | Pupovac | 1896 | None | Left lobe contained tumor | No | Yes | No ref. |
| 6 | Swoboda | 1896 | Same as case 1 | No ref. | No | Yes | No ref. |
| 7 | Bostroem | 1896 | None | Displaced right lobe | No ref. | No | No ref. |
| 8 | Dentler | 1902 | Part of cysts and lumina in tumor called degenerated primitive thyroid tissue | Entire gland replaced by tumor | Yes | No | No ref. |
| 9 | Carter | 1903 | No ref. | No ref. | No | No | No ref. |
| 10 | Wiesinger | 1904 | No ref. | No ref. | No ref. | Yes | No ref. |
| 11 | Poult | 1905 | Fetal and adult thyroid tissue in tumor | Displaced | No | Yes | No ref. |
| 12 | Flesch and Winternitz; first case | 1905 | None | Displaced left lobe | No ref. | Yes | Superior thyroid artery tied in removing part of tumor |
| 13 | Herb | 1906 | Thyroid follicles in capsule and compressed just beneath capsule in tumor | Displaced left lobe | No ref. | Yes | No ref. |
| 14 | Lurje | 1908 | One colloid-filled cyst in tumor | No ref. | No ref. | No | No ref. |
| 15 | Niosi | 1908 | No ref. | No ref. | No ref. | Yes | No ref. |
| 16 | Hunziker | 1909 | None | Displaced right lobe | Yes | No | Superior thyroid artery on right entered tumor |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 17 | Kimura | 1910 | Embryonic thyroid gland-like tissue in fibrous tissue lamellae of capsule | Displaced entire gland | No ref. | No | No ref. |
| 18 | Schönberg | 1911 | None | Left lobe taken with tumor, but not mentioned again | No | No | No ref. |
| 19 | Russell and Kennedy | 1913 | Atypical large cystic areas, supposedly thyroid tissue | Tumor in place of thyroid gland | Yes | No | No ref. |
| 20 | Ehlers | 1914 | Lumina lined with cuboidal epithelium and filled with red material—thought to be thyroid tissue | Attached by a fibrous tissue stalk to right lobe | No ref. | Yes | No ref. |
| 21 | Dorner | 1915 | Necrotic glandular tissue, thought to be thyroid gland | Displaced | Yes | No | No ref. |
| 22 | van Rey | 1916 | None | No ref. | No ref. | Yes | No ref. |
| 23 | Fritzsche | 1920 | No thyroid tissue but glial tissue present in tumor* | Tumor in left lobe | No ref. | Yes | Left superior thyroid artery tied during removal of tumor |
| 24 | Hadda† | 1921 | Yes | Tumor fastened to left side of trachea by cord of tissue, apparently thyroid gland | No | Yes | No ref. |
| | Koerner† | 1922 | None | No ref. | No ref. | Yes | No ref. |
| 25 | Hördemann | 1925 | None | No ref. | No ref. | Yes | No ref. |
| 26 | Tammann | 1925 | Strands of thyroid tissue in capsule separated from tumor by fibrous tissue | Yes | No | Yes | No ref. |
| 27 | Custer | 1926 | Thyroid tissue in fibrous capsule of tumor | Yes | No | Yes | Left superior thyroid artery cut in resection of tumor |
| 28 | Lecène and Mouchet (a) | 1928 | None | Yes | No ref. | Yes | No ref. |
| 29 | Lecène and Mouchet (b) | 1928 | None | Tumor originating from isthmus of thyroid gland | No ref. | Yes | No ref. |
| 30 | Saphir | 1929 | None | No thyroid gland present in fetus—tumor in its place | No | No | Superior thyroid arteries entered tumor |

* See text of article.
† Both authors reported the same tumor. See text.

TABLE II

*Summary of Cases Reported Since 1929 and Additional Earlier Cases Not Included in Saphir's Review*

| No. | Author | Year | Age | Sex | Thyroid tissue present in tumor | Brain present in tumor | Displacement of thyroid gland | Hydramnios present at birth | Operation performed | Thyroid arteries entering tumor |
|---|---|---|---|---|---|---|---|---|---|---|
| 31 | Munker | 1898 | | | | | | | | |
| 32 | Hagenbach-Burckhardt | 1899 | | | | | | | | |
| 33 | Weyl* | 1900 | 10 mos. | | | | | | | |
| 34 | Schneider† | 1903 | Stillb. | F | No | No ref. | Yes | No ref. | No | Superior and inferior thyroid arteries |
| 35 | McGregor and Workman | 1906 | 3 wks. | F | No | No | Yes | No ref. | Yes | Two arteries entered capsule; branch of lingual or facial artery(?) |
| 36 | Ficheaux‡ | 1908 | 4½ mos. | No ref. | No | No ref. | No ref. | No | Yes | No ref. |
| 37 | v. Khautz (jun.) | 1910 | 4 mos. | No ref. | No | Yes | Yes | No ref. | Yes | No ref. |
| 38 | Pellegrini† | 1913 | Stillb. | No ref. | No | Yes | Yes§ | Yes | No | No ref. |
| 39 | Ribbert† | 1916 | No ref. | No ref. | No ref. | Yes | No ref. | No ref. | No | No ref. |
| 40 | Satanowsky† | 1922 | 1½ mos. | No ref. | No | Yes | No ref. | No ref. | Yes | Vascular pedicle |
| 41 | Fessler | 1924 | Newb. | M | No | No | Yes | No ref. | No | No ref. |
| 42 | Bell, J. W.† | 1926 | Stillb. | M | No ref. | No ref. | Yes | Yes | No | No ref. |
| 43 | Brokate | 1928 | 2 days | No ref. | No | Yes | Yes | No ref. | Yes | No ref. |
| 44 | Bell, L. P. | 1930 | 9 mos. | M | No ref. | Yes | Yes | No ref. | Yes | No ref. |
| 45 | Fèvre and Pavie | 1931 | 25 days | F | No ref. | Yes | Yes | No ref. | Yes | Vascular pedicle |
| 46 | Tavares and Gonçalves de Azevedo | 1932 | Newb. | M | Yes‖ | Yes | No | Yes | No | No ref. |
| 47 | Colloridi† | 1933 | 5 hrs. | M | Yes¶ | Yes | Yes | No ref. | No | No ref. |
| 48 | Petersen | 1933 | Stillb.** | F | No | Yes | Yes | Yes | No | No vascularity |
| 49 | Krech | 1933 | 13 mos. | No ref. | No ref. | No ref. | Yes | No ref. | Yes | No ref. |
| 50 | Simões and Auvray | 1934 | Newb. | No ref. | Yes # | No ref. | No ref. | Yes | No | No ref. |
| 51 | Pusch and Nelson | 1935 | Stillb.†† | No ref. | No | Yes | Yes | No ref. | No | No ref. |
| 52 | Tomassini | 1938 | 3½ years | M | No | Yes | On left side of thyroid gland | No | Yes | No ref. |
| 53 | Potter | 1938 | Stillb. | F | No | Yes | Yes | No | No | Small vessels entered capsule |
| 54 | St. George Wilson | 1939 | Stillb. | F | No | Yes | Yes | Yes | No | No ref. |
| 55 | Trillat and Notter | 1939 | Newb. | No ref. | Yes | Yes | Yes | No | No | No ref. |
| 56 | Peter | 1940 | Newb. | M | Yes | Yes | No connection | No | Yes, at 10 wks. | No ref. |
| 57 | Chapman | 1941 | 9 days | M | No | Yes | Yes | Yes | No | No ref. |
| 58 | Magnin | 1942 | | | | | | | | |
| 59 | Sutton and Gibbs | 1944 | 3 mos. | F | Yes | Yes | Yes | No | Yes | No ref. |
| 60 | Munro and Waldapfel | 1944 | 4 wks. | M | Yes | Yes | Yes | No | Yes | No ref. |
| 61 | Marescot Iglesias | 1945 | | | | | | | | |

* From Ewing's "Neoplastic Diseases."
† Collected by Pusch and Nelson.
§ No trace of thyroid tissue found in neck.
‖ Actually called "embryonic endocrine tissue" by the authors.
# "Embryonal thyroid."
** Six month fetus.

1929 and Pusch and Nelson in 1935. In these later articles there are several minor points that might be clarified. In Saphir's analysis the cases of Hadda in 1921 and Koerner in 1922 were presented as two separate tumors. However, Koerner stated that the tumor he was presenting was first published by Hadda in 1921 after which the tumor was given to Koerner for histologic study by Mathias of Breslau. It should be mentioned that although these two authors wrote about the same tumor, they differed as to whether it contained microscopic thyroid tissue. Hadda discerned its presence, while Koerner found no trace of this type of glandular tissue in the tumor. Saphir, in one of his charts, stated that the teratoma of Fritzsche (1920) did not contain brain-like tissue, whereas the latter's original article asserted the reverse. Bell, in 1926, and Pusch and Nelson, in 1935, mentioned a thyroid teratoma reported by Gardner in his "Iconograms" of 1913. Search of that volume has revealed mention of only one teratoma, which proved to be described and pictured as a teratoma of the side of the face above the level of the mandible. Pusch and Nelson, also, included in their review as a probable teratoma of the thyroid gland, the tumor reported by Bell in 1926. Bell's histologic description is rather indefinite, but the photographs and photomicrographs shown would seem to warrant its acceptance as a probable teratoma of the neck in the region of the thyroid gland.

Since these tumors seemingly have their origin from totipotent cells, no one tissue can be used as a label of their individuality, as was suggested by Saphir in regard to thyroid tissue. Hence, for naming these tumors, we must rely on anatomical position as well as on admixture of tissues. One feature that may be employed for this purpose is the relationship of the thyroid arteries to the tumor. However, since only 6 of the 56 tumors have been recorded as supplied by the inferior and/or the superior thyroid arteries, this relationship is of small significance. These vascular connections suggest, at least, that the tissue in these individuals that should have become thyroid gland, developed into the teratomata. It would seem that these cases were true teratomata of the thyroid gland itself. A broader, if not quite so striking, basis for recognition is the more constant tumor-thyroid relationship as noted in the literature. In 44 of the 56 cases all or part of the thyroid gland was replaced by the tumor. In the other 12, save one, no reference was made to the thyroid gland or to other positive relationships. This replacement of thyroid tissue is not pressure displacement of the gland, as is seen in the other tissues of the neck; on the contrary, there is an actual absence

* From Ewing's "Neoplastic Diseases."
† Collected by Pusch and Nelson.
§ No trace of thyroid tissue found in neck.
‖ Actually, called "embryonic adipose tissue" by the authors.
# "Embryonal thyroid."
** Six month fetus.

of part or all of the thyroid gland with tumor occupying its place. It may be surmised that the tumor grew from embryonic tissue destined to become thyroid gland, or at least from immature tissue closely associated with the developing gland. Since other teratomata of the body, for example sacral and testicular teratomata, are named by their anatomical relationships, the 38 tumors which displaced the thyroid gland but are not supplied by the thyroid arteries may be captioned teratomata of the neck in the thyroid region.

Three classifications thus emerge, teratomata of the thyroid gland (6 cases), teratomata of the neck in the region of the thyroid gland (38 cases), and teratomata of the neck probably in the region of the thyroid gland (12 cases).

The identification of adult thyroid tissue in these tumors is unusual, for only 6 of the 56 writers stated it to be present. This is somewhat at variance with Saphir's impression, since he accepted several more of the tumors which he reviewed as containing thyroid tissue. The observations of the various authors collected by Saphir as to the appearance of thyroid tissue in their tumors are offered in Table I.

A surgical procedure had been performed in 29 of the 56 recorded cases. Schimmelbusch, in 1894, carried out the first operation of this type and the patient apparently was cured. The follow-up period in that case, as in the majority of the later cases, was quite short: 25 of the operations resulted in presumed cures, and 4 of the surgically treated patients died. Thus, Pupovac's patient (1896) died suddenly the afternoon of the day of operation. The other fatalities noted were those of van Rey (1916), Fritzsche (1920), and Hadda and Koerner (1921). The cause of death in these cases was either unknown or unmentioned except by Fritzsche. His case was that of a 41-year-old female who died 1 month following operation of widespread metastatic sarcoma, probably originating from the teratoma. So, 25 of the patients treated surgically were relieved of their tumors (44 per cent of cases). A survey of the remaining 27 cases shows the following: (1) 2 dead born fetuses, (2) 10 stillborn infants, (3) 13 newborn children dead within the first few days of life, and (4) 2 cases alive beyond the newborn period. In the last category were Carter's patient (1903), who died at 1 month of age of suffocation produced by progressive enlargement of the tumor, and Lurje's patient (1908), a 53-year-old woman, who died of metastatic sarcoma from a probable malignant proliferation of the teratoma. Thus only one patient in this group who lived long enough to be a fair surgical risk was not treated surgically. The majority of the newborn, class 3 above, died before surgical treatment could be instituted. It might, therefore, be

considered that if the patient is a fair surgical risk this tumor can be removed with a good result.

Hydramnios is commonly associated with teratoma of the neck. This was first observed by Dentler in 1902. Since then, 10 other writers have mentioned it, an incidence of 19.6 per cent, whereas the natural occurrence is 0.5 per cent (DeLee and Greenhill). However, hydramnios is not infrequent with many types of congenital anomalies.

Of all 56 tumors only 3 (Pupovac, 1896; Lurje, 1908; Fritzsche, 1920) proved to be clinically malignant.

With reference only to the teratomata not found in Saphir's study, 19 of the 26 were composed partially of nervous tissue. The sexes were represented nearly equally, and all of these neoplasms were discovered at birth.

The 4 cases follow.

*Case 1*

Baby girl O., a 7 months premature white infant, was delivered at St. Luke's Hospital, Kansas City, on July 18, 1948. The mother was a 20-year-old primipara who had had a normal pregnancy. The past and family histories were without note. Labor was unusual only in its early onset, the estimated date of confinement being October 25, 1948, and in the discovery of hydramnios at birth, the fluid being estimated at 5 l.

A large, soft, irregularly lobulated, cystic mass, measuring 12 by 10 cm., was seen to occupy the anterior right side of the neck (Fig. 1). The child did not respond to stimulation and died 7½ hours after birth, apparently of respiratory failure.

*Gross Description.* Significant necropsy findings consisted of partial atelectasis of both lungs and the tumor of the neck. This was a large mass in the anterior cervical region. It extended from the chin downward and outside the anterior thoracic wall to the level of the xyphoid bone. On the right it reached to the ear and on the left for 3 cm. beyond the midline. Posteriorly, the tumor rested on and partially surrounded the larynx, trachea, and esophagus. It was overlaid by skin which was easily dissected away, and was well encapsulated. The band muscles of the neck were stretched over the anterior and lateral surface of the tumor, and some were very firmly attached to the capsule of the mass. The left lobe of the thyroid gland was loosely attached by fibrous tissue to the posterior surface of the teratoma. The isthmus and right lobe could not be found. Careful dissection revealed that the right superior thyroid artery which was some five times the size of its mate, ended in the superior posterior capsule of the tumor. No other definable vessels were seen to enter the mass. Nerves associated with the tumor were those innervating the band muscles of the neck. The vagus and other large nerves and vessels traversing the neck were displaced laterally. The thymus was not involved in the tumor.

The tumor measured 12 by 12 by 10 cm., and consisted of a large, roughly pear-shaped, nodular, partially cystic and partially solid mass. Section revealed multiple, irregular, cystic spaces varying from 3 mm. to 3 cm. in diameter, with intervening irregular masses of soft to firm grayish white tissue. Some of the cysts contained a clear yellow, water-like material and others contained blood-stained fluid. Several polypoid masses of grayish white soft tissue projected into two of the larger cavities. Cartilaginous and bony areas were found in the center of the tumor.

*Microscopic Description.* This tumor presented a ground substance of stellate cells of various sizes and shapes, surrounding numerous cystic structures lined by various types of epithelia. The types found were squamous epithelium, simple and stratified, the latter showing in one area skin-like structure with an embryonic hair shaft; simple columnar epithelium of goblet type; pseudostratified ciliated columnar epithelium; simple cuboidal epithelium, a portion pigmented and part ciliated; and transitional epithelium, some of which appeared to be ciliated. In some of the cysts the epithelium showed a rapid transition from one type to another. Both striated and smooth muscle cells were present in the tumor plus many areas of cartilage and bone, some of the latter appearing to be developing from cartilage. The fairly well developed bone showed bone marrow. Definitive glandular organization appeared in the tumor, with bands of smooth muscle surrounding lumina lined by columnar epithelium of goblet type. Considerable quantities of what appeared to be embryonic mesenchyme were present, and there was well developed fat. Elastic and collagenous connective tissues could be seen scattered throughout the tumor.

The van Gieson and Masson trichrome stains demonstrated many areas of stellate cells which had the characteristics of neuroglia. Several areas of the tumor, separated from the surrounding connective tissue by fibrous bands, showed slit-like spaces bordered by many layers of small dark-staining cells giving the appearance of neuro-epithelial canals. Filamentous folds of pigmented and unpigmented cuboidal epithelium which enclosed connective tissue cores containing wide capillary-like vessels were found in the tumor. These areas had the appearance of epithelium of the ciliary body and choroid plexus. Another area took the form of ependyma. Adult multipolar neurons were recognized in one of the sections. Capillaries and arterioles were noted.

Thyroid tissue was not observed.

### *Case 2*

Baby girl F. was a 6 months stillborn fetus delivered on June 5, 1929, at St. Luke's Hospital in Kansas City. The mother of this infant was a 30-year-old white primipara. The past and family histories were without note. The last menstrual period had been November 20, 1928. The patient was admitted in labor and was delivered without difficulty by breech assist on the second hospital day. A large amount of amnionic fluid was noted.

*Gross Description.* At necropsy complete lack of pulmonary aeration and the tumor in the neck were the only significant findings. A large, irregularly lobulated mass was seen to lie in the left anterior region of the neck (Fig. 2). It extended from the zygoma to the clavicle, and from the trachea to the posterior border of the mastoid process. The tumor was loosely covered by skin which was necrotic over an area measuring 2 cm. in diameter on the summit of the tumor. The tumor was well encapsulated, and posteriorly it rested upon the inferior surface of the floor of the mouth and the deep structures of the neck which were displaced as if by expansile growth. It protruded superiorly and anteriorly over the external surface of the mandible and elevated the left ear and the skin of the cheek. The band muscles were reduced to thin ribbons attached to the fibrous tissue capsule of the tumor. The teratoma was fastened firmly only at its medial border to the left side of the tracheal cartilages. The right lobe and isthmus of the thyroid gland were identified, but the other lobe was absent. On the left the common carotid artery was approximately four times the length of its counterpart, and appeared to be stretched over the lateral surface of the tumor. The inferior thyroid artery from the thyrocervical branch of the left subclavian artery and the superior thyroid artery from the common carotid arose in their usual places and entered the tumor on the medial and lateral aspects, respectively. The nerves crossing this side of the neck were pushed lateralward. The thymus was not associated with the tumor.

The tumor measured 10 by 12 x 8 cm., and consisted of a semioval, partly solid, partly cystic, grayish black mass. Multiple cuts through it revealed a heterogeneous parenchyma with cystic structures measuring up to 2 cm. in diameter, lined by smooth and papillary membranes.

*Microscopic Description.* Only a limited number of blocks were available for study. Here again, however, were seen the great diversity of tissues, the background of loosely knit stellate cells bordering many cyst-like spaces of various sizes, shapes, and epithelial linings, these last being squamous, simple and stratified; simple columnar; cuboidal; and transitional. Both smooth and skeletal muscle fibers were seen, and there were considerable embryonic and adult cartilage and collagenous

connective tissue. Neuroglia was demonstrated with Masson and Bielschowsky stains.

No thyroid tissue was seen in the tumor.

### *Case 3*

Case 3 was a stillborn, somewhat macerated infant. No additional history was available.

*Gross Description.* The teratoma replaced almost all of the structures of the neck. The relationship of the tumor to the thyroid arteries could not be determined because of the deterioration of the tumor, and a separate thyroid gland could not be identified. The position of the tumor made it seem most likely that it was derived from the thyroid gland. No further gross description was obtained.

*Microscopic Description.* This tumor was similar to the ones just described and contained nearly the same types of epithelia as in case 1. Here again were seen smooth and skeletal muscle; cartilaginous, myxoid, collagenous, and elastic connective tissues; and capillaries and arterioles. A rather markedly different type of epithelium appeared here than had been seen in the other tumors. It consisted of an inner simple cuboidal epithelium overlaid with a syncytium of a single cell thickness, the whole having the characteristics of trophoblastic epithelium. Throughout the sections there were clumps of deep-staining polygonal cells with a finely granular cytoplasm and vesicular nuclei. Groups of small alveolar structures lined with cuboidal epithelium were crowded together to appear like glands of the mucous type. Organoid structures were better developed here than in the other tumors studied, one cystic structure presenting an epithelium of columnar cells with well formed thick bands of smooth muscle lining the cyst walls. Neuroglia-like tissue was identified with special stains.

No evidence of thyroid tissue was found in the sections studied.

### *Case 4*

J. M. was a 2-months-old white male who was admitted to the University of Kansas Hospital in Kansas City, Kansas, on February 25, 1940, for removal of a tumor of the neck. The mother of the child was a young primipara who had no difficulty with her pregnancy or delivery. The child weighed 7 lbs. at birth, and at that time a large mass was noted on the left side of the neck. The mass produced dysphagia and had been aspirated repeatedly for the relief of this symptom prior to admission. During the first 2 months of life the tumor spread anteriorly and downward toward the right side. The child gained slowly and weighed 9½ lbs. on admission. Physical examination at that time revealed a tense, multilocular, cystic mass, 12 by 12 cm. in diameter, overlying the anterior left cervical area and extending across the midline to the right for a short distance.

On February 29, 1940, the tumor was removed by the late Dr. Earl C. Pagett.

He commented in the operative note that ". . . a line of cleavage was found in the fascia of the neck and the tumor shelled out with the finger." The child recovered from the operation without undue difficulty and was discharged on March 20, 1940, without symptoms.

Nothing is known of the later course, for contact with the family has been lost.

*Gross Description.* The tumor was an irregular mass measuring 15 by 9.5 by 5 cm. and weighing 310 gm. (Figs. 3 and 4). The external surface was bosselated, well encapsulated, and roughened by numerous fibrous tags. On sectioning, the surface was cystic and showed a varigated appearance, there being numerous, friable, light gray, cellular areas intermingled with cystic structures containing a viscid clear or bloody fluid and small areas of cartilage. A few calcific deposits were scattered throughout.

*Microscopic Description.* In structure this tumor was much the same as the other three. It showed most of the same types of epithelia, and here again were seen fatty, bony, cartilaginous, collagenous, and fibrous connective tissues; smooth and skeletal muscle; and capillaries and arterioles. In one portion of the tumor were seen closely arranged acini lined with columnar cells with basally located nuclei and granular red cytoplasm, and in another was a well defined area of enchondral ossification. Organoid spaces lined by various types of epithelium were seen, surrounded by walls containing smooth muscle bundles. Here again the epithelium of these lumina changed rapidly from one type to another. In another portion of the tumor there was an area, set aside from the usual type of tissue by fibrous capsule, which was made up of adult neuroglial cells containing numerous well defined ganglion cells plus a few, rounded, dark purple, laminated, calcified spherules which had the appearance of psammona bodies. An ependyma-like structure bordered one edge of this section.

Thyroid tissue was not identified in this tumor.

## Discussion

The first two tumors (cases 1 and 2) are definitely teratomata of the thyroid gland because of their anatomical relationships and blood supply. A fibrous tissue capsule separated each tumor from what remained of the thyroid gland.

The last two tumors (cases 3 and 4) are not so well defined, for proof of their anatomical relationships could not be obtained. It can only be supposed that they were in the thyroid region, a supposition which in fact cannot be defended. Therefore, these two can be designated as teratomata of the neck, probably in the region of the thyroid gland. Tissue that appeared to be neural in origin and form was found

in all four tumors, but no adult thyroid tissue could be made out. No evidence of malignancy was observed.

The birth of the first two infants was attended by hydramnios, but it is not known whether this occurred in the latter two cases. Case 4, J.M., underwent operation and was relieved of his tumor. Two infants were stillborn and one was not operated upon because of its extremely poor condition.

## Summary

Fifty-six cases of teratomata of the thyroid gland and teratomata of the neck in the region of the thyroid gland have been assembled from the literature and summarized. Only 6 of the tumors can be called true teratomata of the thyroid gland, being the ones in which the thyroid arteries supplied the tumor. Thirty-eight more were definite teratomata of the neck in the region of the thyroid gland because they replaced all or part of the gland. The remaining 12 are called teratomata of the neck, probably in the region of the thyroid gland, because of their general appearance. Twenty-nine of the tumors have been removed surgically, 25 apparently with good results. Hydramnios is commonly found associated with this abnormality. The presence of brain tissue, the sex and age incidence, and the malignant properties of these tumors have been reviewed. Two new cases of teratomata of the thyroid gland and two cases of teratomata of the neck, probably in the region of the thyroid gland, are added to the literature.

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## DESCRIPTION OF PLATE

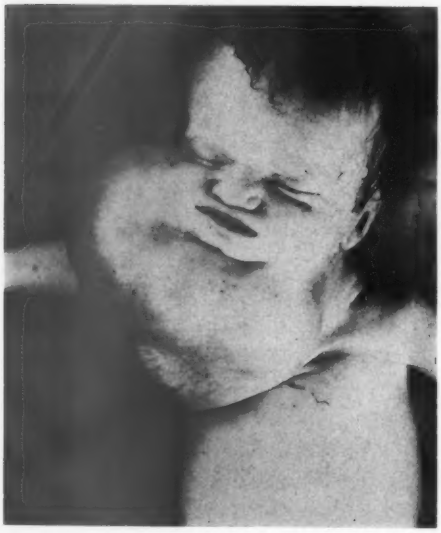
### PLATE 83

FIG. 1. Case 1. Gross photograph of teratoma of baby girl O.

FIG. 2. Case 2. Gross photograph of baby girl F., showing the tumor in place in the neck.

FIG. 3. Case 4. Gross photograph of J. M. with tumor before surgery.

FIG. 4. Case 4. Gross photograph of J. M. after removal of tumor on day of discharge from hospital.



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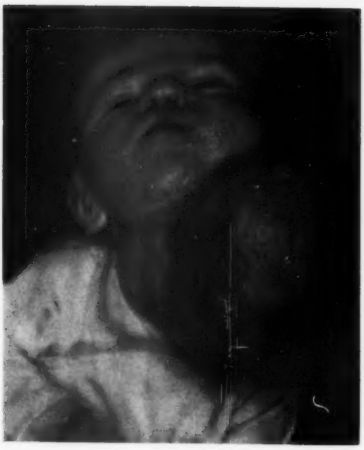

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Bale Cervical Teratoma

# AMYLOID AND MYELOMA *

DAVID C. DAHLIN, M.D., and MALCOLM B. DOCKERTY, M.D.
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Patients suffering from myeloma are prone to the development of deposits of amyloid in various sites. The distribution of this peculiar material in myeloma is often similar to that observed in primary systemic amyloidosis, thus necessitating a separate category for the amyloid deposition that is associated with myeloma. Not so generally known, however, is the fact that deposits of amyloid may occur also within the nodular proliferations of the myeloma cells themselves. To go further and maintain that the presence of amyloid in a tumor, the nature of which is debatable, is almost diagnostic of the myelomatous nature of that tumor, has not been suggested. Our evidence for such assumption serves as the chief basis for this paper. The more prosaic aspects of amyloid with myeloma will be relegated to the background.

The occurrence of amyloidosis in a patient with myeloma was first reported by Adams and Dowse[1] in 1872. Magnus-Levy,[2,3] in 1931 and 1933, reported the results of a review of 150 cases of myeloma, in 29 of which significant amounts of amyloid were found. We have found 21 additional examples of this condition in the subsequent literature, making a total of 50 cases. In 22 of these 50 cases, deposits of amyloid were observed within the myelomas. In several of these the material occurred as small homogeneous masses in the microscopic sections. Giant cells of the foreign body type surrounded the islands of amyloid in some instances. Gross tumors composed entirely of amyloid occurred in 15 cases. These tumors were usually partially within bone. Severe generalized amyloidosis similar to primary systemic amyloidosis was observed in 11 cases. In 22 there was mild infiltration of various organs. In 8 cases there were coexistent systemic and intramyelomatous deposits. Macroglossia was observed in 10 cases, and subepidermal deposits were noted in 4. Gastro-intestinal involvement was common and in Randall's case[4] it caused intestinal obstruction. In 18 of the 50 cases Bence Jones proteinuria was discovered; in 6 it was specifically stated to be absent.

Myeloma, in this discussion, is used to indicate the primary neoplasms, usually of bone marrow, which are made up of more or less immature cells of the plasma-cell line. Amyloid is defined as a homogeneous amorphous material which stains pink with hematoxylin and

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eosin, metachromatically with methyl violet and methyl green, yellow to pink with the van Gieson connective tissue stain, and pink to red with Congo red. The microscopic features of the condition in the cases to be considered in this paper are given in Figures 1 to 13.

## Necropsy Cases

In reviewing the routine microscopic sections in the 29 cases of myeloma studied at necropsy at the Mayo Clinic prior to January 1, 1947, systemic deposits of amyloid were observed in 3 instances. Two of these cases have been reported previously.

The third case was that of a man, aged 75 years, who complained of pains about the joints of his extremities and dyspnea on exertion. The erythrocytic sedimentation rate was 78 mm. in the first hour (Westergren). There was no Bence Jones proteinuria although albuminuria varied from grade 1 to grade 3 (on the basis of 1 to 4). The patient died suddenly, and necropsy disclosed calcareous aortic stenosis together with marked deposition of amyloid in the walls of the small and medium-sized arteries and veins of all of the organs of the thorax and abdomen. The diaphragm and thyroid gland were similarly affected. The deposits of amyloid were principally in the media, but the lumina of involved vessels were often greatly narrowed (Fig. 12). An amyloid tumor measuring 4 by 2.5 by 1 cm. was found in the posterior wall of the hypopharynx. Unsuspectedly, typical myeloma cells were found in the two blocks of bone marrow that had been saved.

This case illustrates the necessity of searching for myeloma when unusual deposits of amyloid are found. Interestingly enough, none of the specimens studied in the 29 cases demonstrated amyloid within the myeloma tissue. In an additional case of myeloma, for the necropsy findings in which we are indebted to Dr. H. E. Taylor of Vancouver, British Columbia, the bone marrow of the ribs, vertebrae, and sternum showed myeloma cells of rather immature type. The marrow of the femur, on the other hand, was made up of fat cells which had, in large measure, been replaced by material which stained pink with hematoxylin and eosin (Fig. 13). The denser portions of this material showed the characteristic special staining reactions of amyloid.

## Surgical Cases

Over a comparatively short period we have had the unique opportunity of making observations in no less than 6 cases of myeloma of bone in which excised material exhibited varying amounts of amyloid. One of these cases was particularly interesting in that the amyloid

component of a rib tumor overshadowed in great measure the nature of the underlying myeloma. The group presented such a striking pathologic picture that we decided to investigate our surgically excised myeloma material in a quest for an amyloid component. Single and multiple osseous and extra-osseous myelomas were all accepted, but those diagnosed by means of sternal aspiration were deleted except in one unusual case (case 12, Table I). This exclusion limited to 65 the total number of Clinic cases studied, but to this group we have been able to add a further example (case 14, Table I) through the courtesy of Dr. R. H. Fenstermacher of Vicksburg, Mississippi.

The original screening was done by use of microscopic sections stained with hematoxylin and eosin. Depending upon the amount of tissue available, the number of blocks sectioned varied from one to ten. In the average case, three sections were examined. Of the 66 cases, the specimens in 13 contained typical deposits of amyloid which were verified in each instance by the use of special stains. In the 14th example (that of Dr. Fenstermacher), material for special stains was not available but the appearance of the sections stained with hematoxylin and eosin was so characteristic that the case was included as an undoubted "positive." In 12 of these 14 tumors the deposits of amyloid appeared as large and small sheets or clumps of homogeneous, amorphous, hyalin-like material in a sea of myeloma cells. In 8 of these the "foreign" nature of the material was emphasized microscopically by the presence of foreign body giant cells in and around the deposits. Some of these cells were seen to contain engulfed amyloid. In 10 of the cases the amyloid was seen in each section examined. In 4 cases (cases 8, 9, 10, and 11, Table I) amyloid was seen in only one section.

Two of the cases in this study deserve special mention. They are similar to one described by Glaus.[5] In case 12 small deposits of amyloid were noted within the myeloma tissue which was obtained by sternal aspiration. In addition the majority of the tumor cells contained intracytoplasmic inclusions which, on careful examination, were found to consist of one or several angular masses of homogeneous material (Figs. 8 and 9). This material stained weakly for amyloid. Some of the cells were distended to two or three times normal size, but they still retained recognizable "myeloma cell nuclei." On cursory examination this condition might be confused with a lipid storage disease. In case 13, biopsy of a lesion in the ilium revealed that approximately 10 per cent of the cells were distended by similar masses of homogeneous material (Fig. 10) which, again, showed the staining reactions of amyloid. In this one there were no intercellular deposits

TABLE I

*Amyloid-bearing Myelomas*

| Case | Age, in years | Sex | Bones affected* | Bence Jones proteinuria | Reaction with special stains | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Methyl violet† | Methyl green† | van Gieson's | Congo red |
| 1 | 45 | M | Rib and adjacent vertebrae | No | + | + | Yellow | Red |
| 2 | 58 | M | Cervical vertebrae | No | + | + | Yellow | Red |
| 3 | 58 | F | Thoracic vertebra | No | + | + | Yellow | Pink |
| 4 | 48 | F | Multiple (sacrum) | No | + | + | Yellow | Red |
| 5 | 55 | M | Thoracic vertebra | No | + | + | Pink | Pink |
| 6 | 70 | M | Left humerus | No data | + | + | Pink | Pink |
| 7 | 62 | M | Multiple (clavicle) | No data | + | + | Yellow | Red |
| 8 | 56 | M | Multiple (clavicle) | No | + | + | Yellow | Red |
| 9 | 60 | M | None (pharynx) | No | + | + | Yellow | Red |
| 10 | 55 | M | Bones of face | No data | + | + | Pink | Pink |
| 11 | 46 | F | Multiple (antrum) | No | + | + | Pink | Pink |
| 12 | 55 | F | Sternum | Yes | + | + | Yellow | Pink |
| 13 | 44 | F | Multiple (ilium) | No | + | + | No data | Pink |
| 14 | 60 | M | Right humerus | No | No tissue available | | | |

* Site of excision for biopsy is indicated in parentheses.

† Plus sign means that the amyloid stained metachromatically.

of amyloid. Again the question of lipid storage disease was considered by one of the pathologists who reviewed the specimens.

In the group of 14 cases in which results were positive, the disease was obviously disseminated in 7 instances. In 6 the myeloma presented as a solitary tumor; however, proof is lacking that no other bones were affected. In case 9 the myeloma was limited to the soft tissues of the pharynx; no bones showed roentgenologic change.

## Comment

In view of the theory championed by Magnus-Levy[2] that amyloid is related in some way to Bence Jones protein, it is interesting that this protein was found in the urine of only one of the patients in the surgical group, although specific tests for it were carried out in 11 cases. This low incidence of Bence Jones proteinuria may be explained in part by the fact that many of these patients exhibited an early, localized phase of the disease when examined at the Clinic. Yet the observation that 20 per cent of the surgical myelomas contained amyloid deposits and that in 2 of them the material was actually within the myeloma cells, strongly supports the theory that plasma cells produce amyloid or some similar precursor substance.

The diagnosis of myeloma in these cases was based on the histologic study of specimens from the neoplasm. In all of them the tumors were composed almost entirely of immature plasma cells having eccentric nuclei and finely granular abundant cytoplasm. The cytoplasmic inclusions in 2 cases have been described above. The presence of large nucleoli and mitotic figures attested to the immaturity of the plasma cells. Binucleated forms were not uncommon. Sternal aspiration was performed in but 2 of these cases. In case 13 it was the only source of material available. The low incidence of sternal aspiration in this group may be explained by our method of selection of cases and by the fact that diagnosis was established by biopsy material from the various sites. In addition, some of the patients were seen before the value of sternal aspiration was established. Knowledge of the presence or absence of myelomatous involvement of sternal marrow would have been of prognostic significance in those patients who appeared to have solitary tumors.

The results of follow-up studies on this surgical group were those to be expected in myeloma. Eight of the patients died 6 months to 4 years after the diagnosis was made. Five are still suffering from their disease. The patient with a pharyngeal tumor had enlarged cervical lymph nodes but no demonstrable osseous lesions. He was alive and

well 12 years after surgical removal of the tumor supplemented by local irradiation therapy.

The absence of intramyelomatous amyloid in the 29 necropsy cases studied contrasts sharply with the high incidence in the surgical specimens. We can offer no adequate explanation for this. The fact that in 12 of the 14 surgical cases the lesions actually presented as tumors with extra-osseous extension may indicate that the presence of amyloid makes the tumors larger than usual. The patients with large tumors are certainly more likely to require surgical treatment.

The pattern of amyloid deposition illustrated by the photomicrographs is so characteristic that when it is recognized, an underlying myeloma should be earnestly sought in the cases in which it is not immediately obvious. To our knowledge similar deposits of amyloid rarely, if ever, occur within other neoplasms. It should be stressed that the nature of material suspected of being amyloid must be confirmed by the use of special staining reactions as illustrated in Table I. The variability of the affinity for Congo red by the amyloid in myeloma is well known. The van Gieson connective tissue stain is of great value in differentiating amyloid from dense collagen and bone which stain a brilliant red.

## SUMMARY

Fourteen examples of amyloid-bearing myeloma were found in a series of 66 cases of surgical myeloma. In addition, brief reference is made to 2 previously unreported cases of multiple myeloma studied in necropsy material, one with systemic amyloidosis and the other with amyloid infiltration of the marrow of the femur.

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

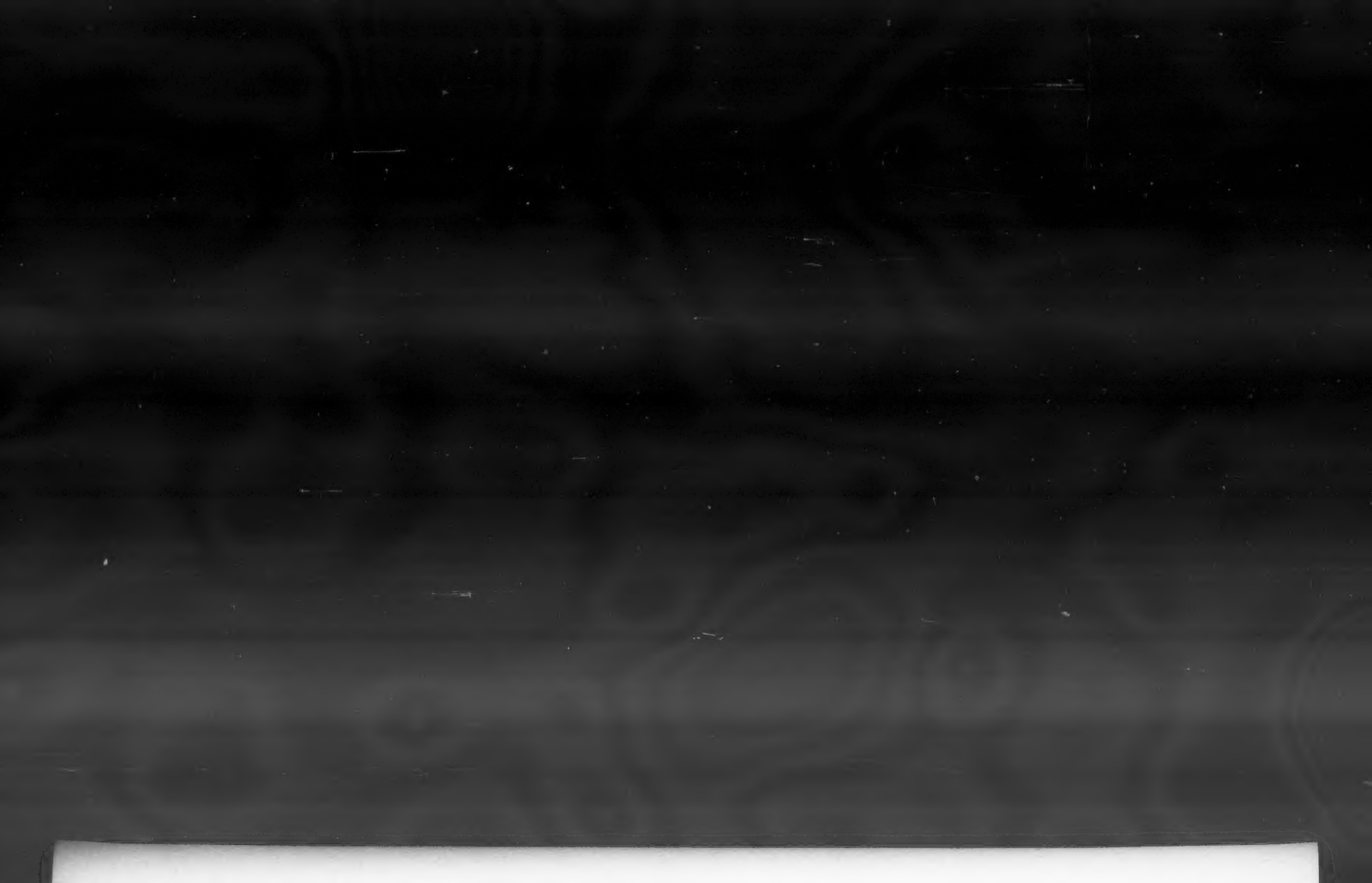
### PLATE 84

FIG. 1. Case 1. Sheets of amyloid in a myeloma showing an especially prominent perivascular distribution. Hematoxylin and eosin stain. × 125. Cases 5 and 7 were very similar to this one.

FIG. 2. Case 2. Myeloma containing small masses of amyloid with foreign body giant cell reaction around them. Hematoxylin and eosin stain. × 175.

FIG. 3. Case 3. Myeloma containing sheets of amyloid and associated foreign body giant cells. Of note is the superficial resemblance to osseous tissue. Hematoxylin and eosin stain. × 125.

FIG. 4. Case 4. Characteristic masses of amyloid surrounded by foreign body giant cells. This tissue was obtained by needle biopsy from a myeloma of the sacrum. Hematoxylin and eosin stain. × 175.



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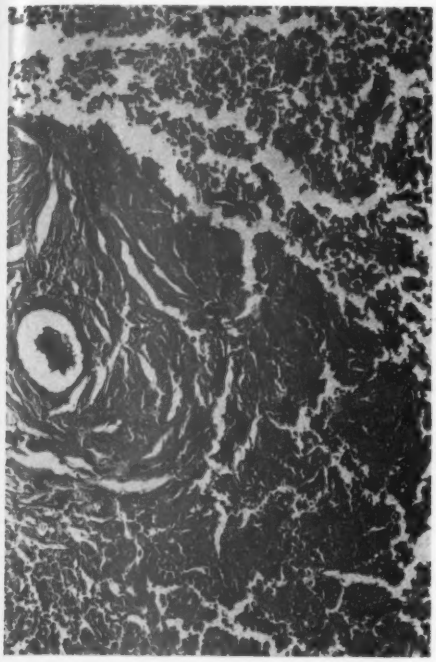

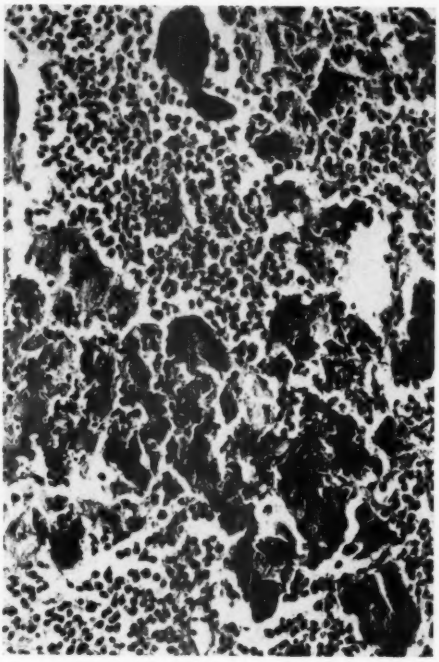

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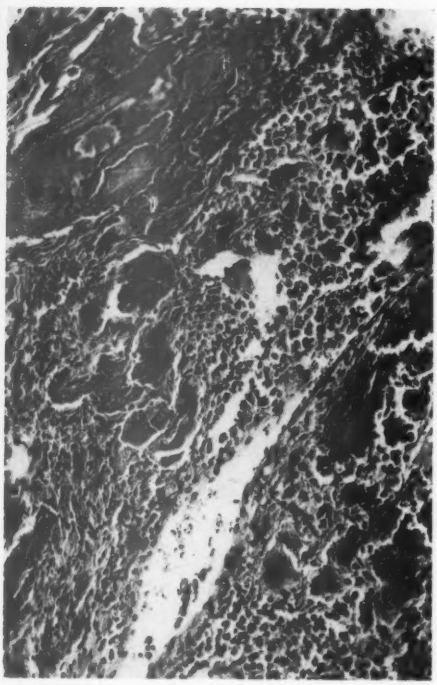
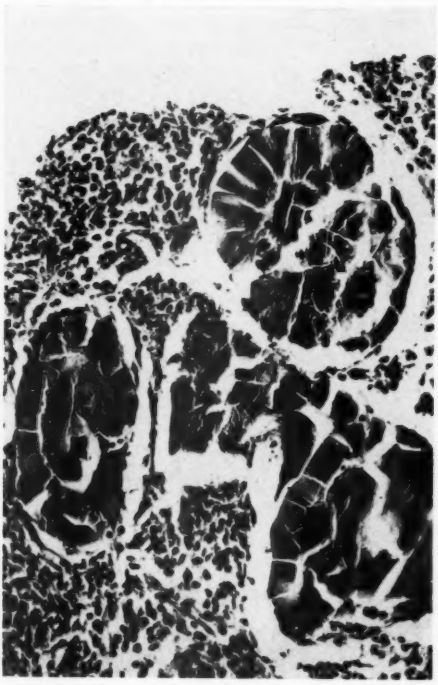




4

Dahlin and Dockerty

Amyloid and Myeloma

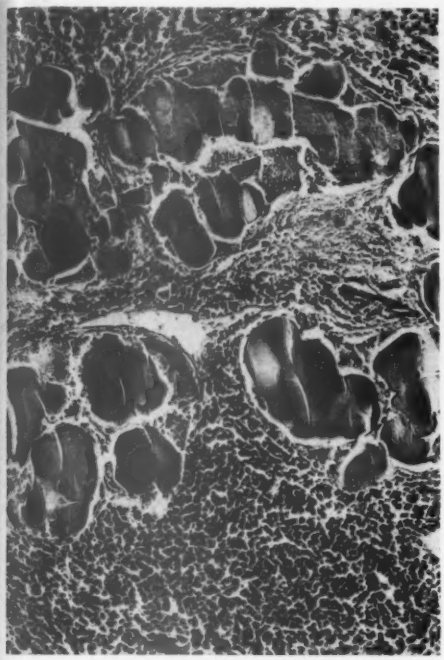
PLATE 85

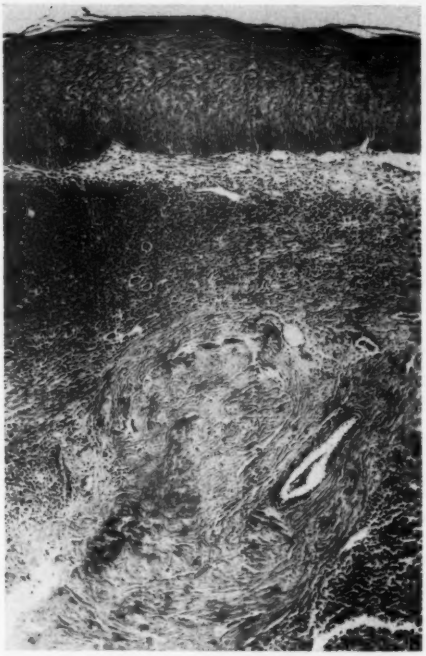
FIG. 5. Case 6. Masses of amyloid partially surrounded by foreign body giant cells. Hematoxylin and eosin stain. × 125.

FIG. 6. Case 8. This was the only accumulation of amyloid observed in this case. Hematoxylin and eosin stain. × 125. Similar isolated deposits were observed in cases 10 and 11.

FIG. 7. Case 9. Amyloid mass within a myeloma of the pharynx. Hematoxylin and eosin stain. × 50.

FIGS. 8 and 9. Case 12. Myeloma cells containing masses of homogeneous material that stained weakly for amyloid. Of note is the rouleau formation. Small intercellular deposits of amyloid were observed in this case. Wright's stain. × 800.





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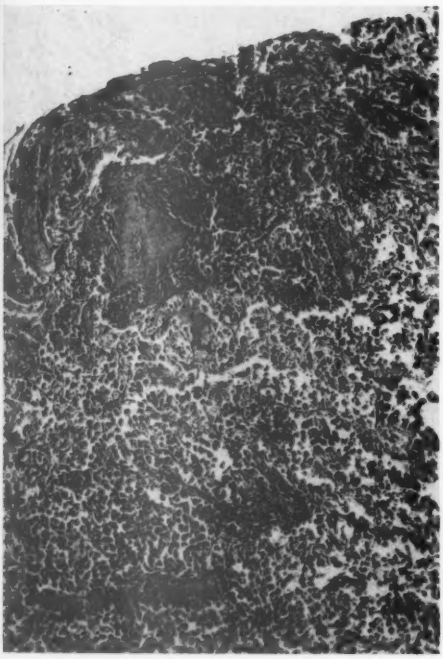

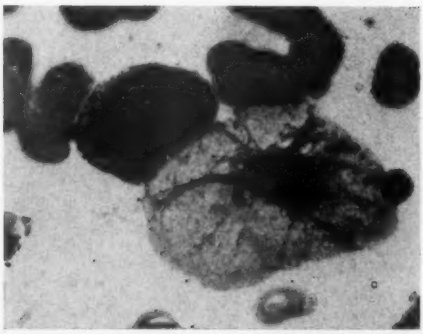

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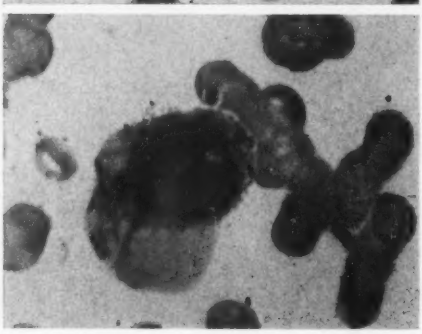


9

Dahlin and Dockerty

Amyloid and Myeloma

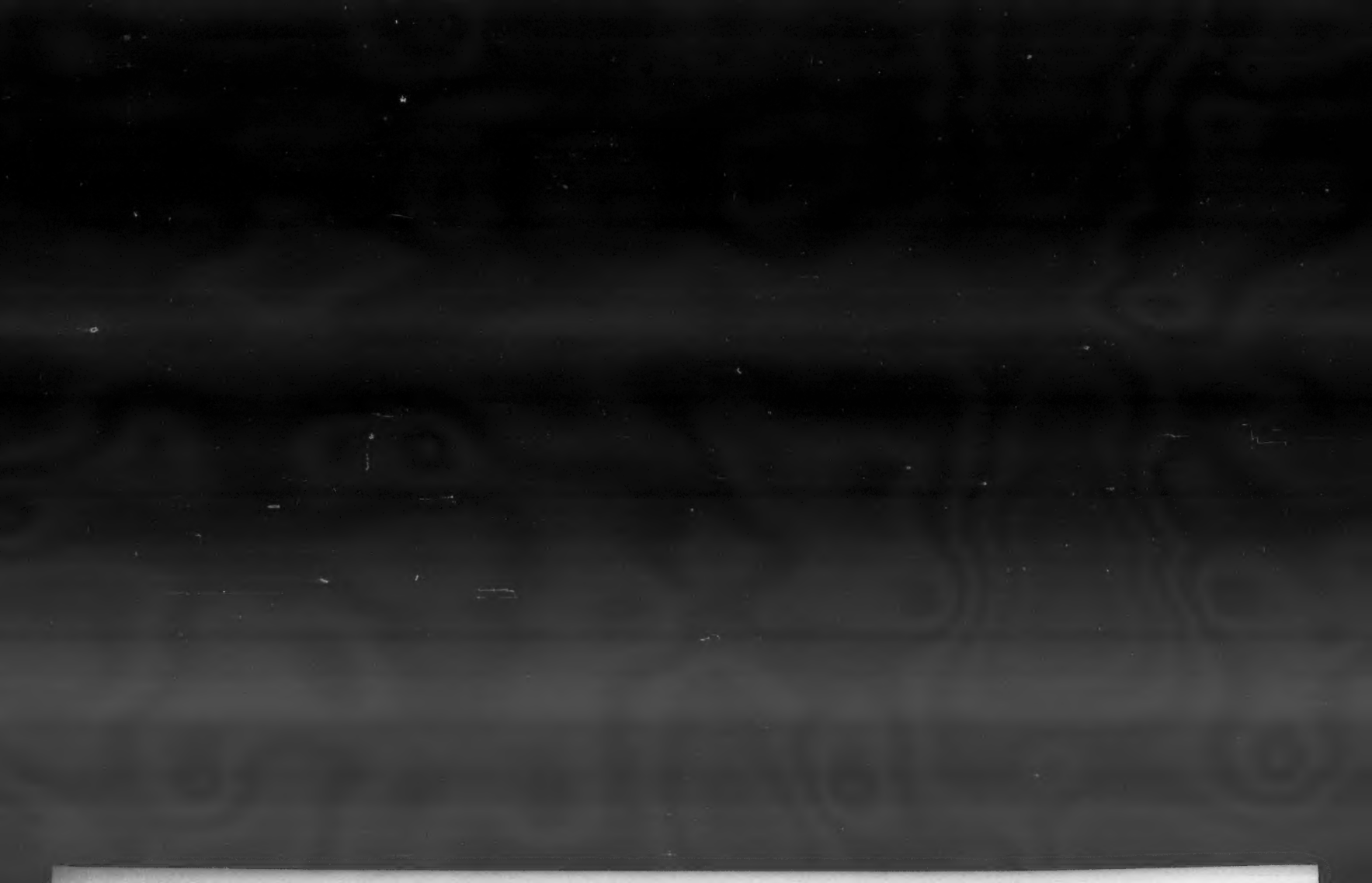
PLATE 86

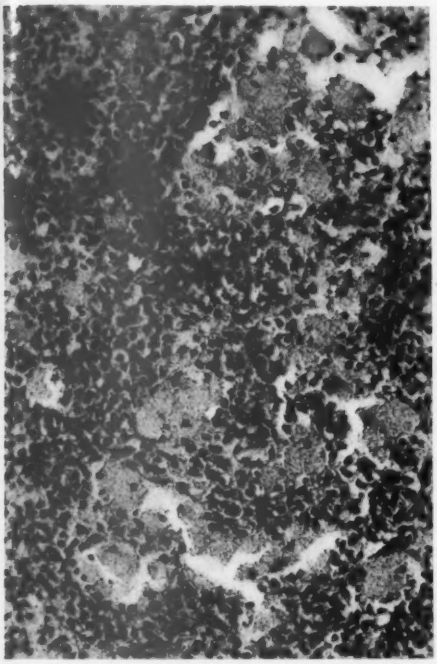
FIG. 10. Case 13. Biopsy of ilium showing some of the cells distended by homogeneous material which stained weakly for amyloid. Hematoxylin and eosin stain. × 175.

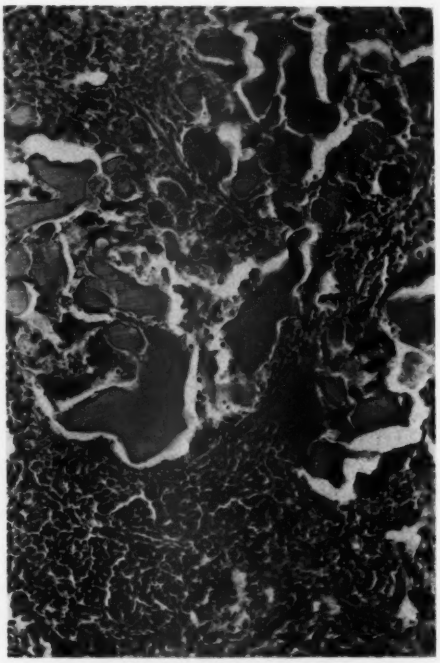
FIG. 11. Case 14. Characteristic deposits of amyloid surrounded by foreign body giant cells in a myeloma of the humerus. Hematoxylin and eosin stain. × 125.

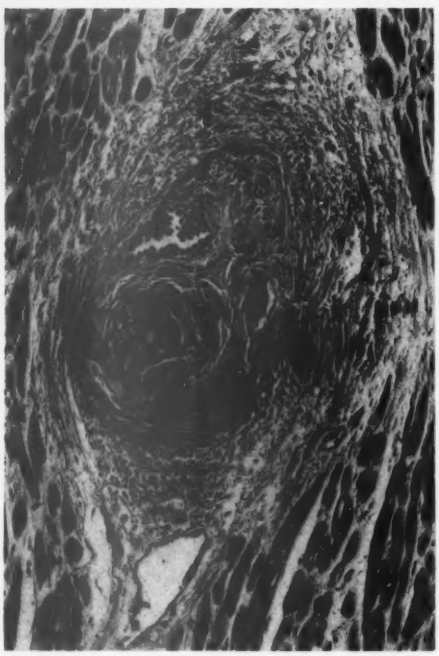
FIG. 12. Mass of amyloid in the wall of a small myocardial vessel in a case of systemic amyloidosis associated with myeloma. Hematoxylin and eosin stain. × 115.

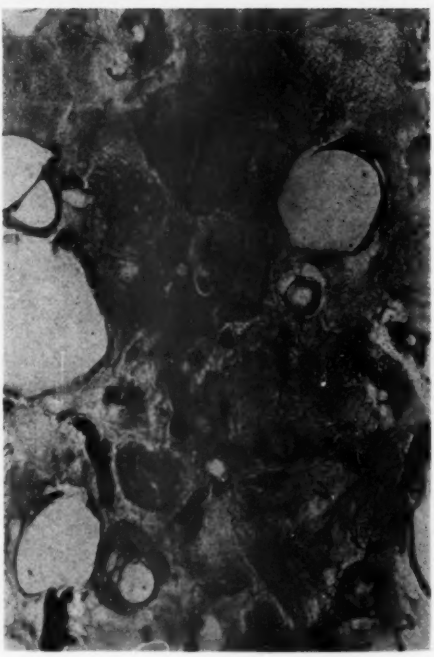
FIG. 13. Amyloid replacement of the marrow of the femur in a case of multiple myeloma. Hematoxylin and eosin stain. × 340.











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Amyloid and Myeloma

# PATHOLOGIC CHANGES INDUCED IN GUINEA-PIGS BY ADMINISTRATION OF DIETS DEFICIENT IN THE ANTI-STIFFNESS FACTOR*

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The observation by Wulzen and Bahrs[1] that maintenance of guinea-pigs upon pasteurized or skim milk diets resulted in stiffness of the wrists initiated a series of studies, primarily biochemical, by van Wagtendonk, Wulzen, and associates[2-15] and by investigators in other laboratories,[16-21] upon guinea-pigs affected by this type of dietary deficiency. It is the purpose of this paper to describe the pathologic anatomy of the condition. For convenience, descriptions are based upon animals maintained at the Lilly Research Laboratories, but are supplemented by observations made on animals at Oregon State College.

In the course of this study, tissues from 662 guinea-pigs maintained at the Lilly Research Laboratories were examined histologically. Of this number, 54 were normal controls, 237 received a deficient diet, and 371 received a deficient diet supplemented with various substances that were being tested for curative or prophylactic action. Some of the 371 received the supplements throughout the experiment, while others were treated for various periods lasting from a few days to several weeks. Some of these died or were killed while still under treatment, and others had received no treatment for weeks or months before death. However, in order to avoid complications, it seems best to omit analysis of the findings in these animals, and to content ourselves with the remark that many developed lesions like those seen in the untreated pigs. The descriptions are based primarily, and specific statements as to the frequency of various lesions are based solely, upon examination of the 237 untreated animals.

Of the untreated animals, 58 were killed while in good physical condition, and 27 were killed when moribund or emaciated. Forty died of pneumonia and 8 of peritonitis. Thus, 106, or 45 per cent, died of an acute infection or were killed before being severely injured by the deficiency. The number of animals dying or killed during 50-day periods on deficient diets follows: 9 after 18 to 48 days; 27 after 51 to 99 days; 46 after 100 to 149 days; 45 after 151 to 198 days; 33 after 206 to 244 days; 24 after 253 to 299 days; and 53 after 301 to 518 days.

* Received for publication, July 1, 1949.

The composition of the diets employed is given in Table I. Twenty-two pigs received diet 1, 200 received diet 2, and 15 received diet 23 or diet 24. Fifteen of those on diet 2 received additional amounts of α-tocopherol. Since no essential difference in lesions in pigs on the various diets was seen, the groups will not be discussed separately.

TABLE I

*Diet No. 1*

| | |
|---|---|
| Pasteurized skim milk | 100 cc. |
| Skim milk powder | 10 gm. |
| Ferric chloride | 0.82 mg. |
| Copper sulfate | 0.78 mg. |

This mixture was fed *ad lib.* and supplemented by the following:
Orange juice, 1 cc./100 gm. of body weight daily.
Carotene (90% β, 10% α), 233γ 3 times weekly (fed in 0.2 cc. Wesson oil).
Iodized salt and autoclaved straw *ad lib.*

*Diet No. 2*

| | |
|---|---|
| Skim milk powder | 16 gm. |
| Water | 84 gm. |
| Ferric chloride | 0.25 mg. |
| Copper sulfate | 0.25 mg. |

To each 90 gm. of this mixture the following synthetic vitamins were added:

| | |
|---|---|
| Thiamine hydrochloride | 0.20 mg. |
| Pyridoxine hydrochloride | 0.10 mg. |
| Calcium pantothenate | 0.10 mg. |
| Riboflavin | 0.50 mg. |
| Nicotinic acid | 1.00 mg. |
| *p*-Aminobenzoic acid | 2.00 mg. |
| Inositol | 10.00 mg. |
| Choline chloride | 50.00 mg. |

The following vitamins were fed daily in 0.25 cc. Wesson oil:

| | |
|---|---|
| Carotene (90% β, 10% α) | 100γ |
| Irradiated ergosterol | 40 I.U. |
| α-Tocopherol (as distilled natural tocopherols) | 0.10 mg. |
| 2-Methyl-1, 4-naphthoquinone | 0.10 mg. |

The animals received also:

| | |
|---|---|
| Ascorbic acid | 50 mg. per week |
| Autoclaved straw and mineral block | *ad lib.* |

| | *Diet No. 23* | *Diet No. 24* |
|---|---|---|
| Purified casein | 20 gm. | 20 gm. |
| Brewers' yeast, dried | 15 gm. | 15 gm. |
| Lard | 10 gm. | 10 gm. |
| Sucrose | 36 gm. | 35 gm. |
| Celluflour | 15 gm. | 15 gm. |
| Salts, no. 1 | 4 gm. | |
| Salts, no. 2 | | 5 gm. |
| Vitamin A concentrate | 1200 USP units | 1200 USP units |
| Vitamin D concentrate | 170 USP units | 170 USP units |
| Distilled natural tocopherols | 8 mg. α-tocopherol | 8 mg. α-tocopherol |
| 2-Methyl-1, 4-napthoquinone | 2 mg. | 2 mg. |

Salts no. 1 provided 0.839 gm. of calcium and 0.205 gm. of phosphorus per 100 gm. of ration. Salts no. 2 provided 1.037 gm. of calcium and 0.436 gm. of phosphorus per 100 gm. of ration.

Diet 1 was the first one employed and was patterned after the earlier diet of the Oregon State College workers,[2] and diet 2 was patterned after their later diet.[4] Diets 23 and 24 were patterned after diets used by Hogan and Regan.[16] It was thought that the latter two diets would not only be deficient in the anti-stiffness factor, but because of the increased calcium and phosphorus content might produce even more calcification.

## Observations

The animals showed much individual variation in response to the dietary deficiency, but they were all smaller than the controls. Some survived for as long as 9 or 10 months without clinical effect other than failure to show optimal growth, whereas others became quite emaciated and showed severe injury within a few months. About one-fourth of the animals showed degrees of testicular atrophy ranging from cessation of spermatogenesis in some tubules to complete loss of spermatogonia from all tubules.

### *Musculo-Skeletal System*

Abscesses often developed in the foot pads and, in general, were largest and most numerous in the thinnest animals. In some cases only a single paw was affected, but usually two or more were involved. In the earlier abscesses the pus had a somewhat mucoid appearance, but in the older ones it was thick. Ulceration over the abscesses occurred sometimes, but destruction of bones of the feet was never encountered. Abscesses were found also about the knees, elbows, and shoulders, intramuscularly at the tip of the scapula, in the intercostal muscles and over the ribs laterally and dorsally, and deep within the muscles of the legs adjacent to the periosteum of the femur or humerus. Abscesses in one or more of these locations occurred in about one-eighth of the animals. The abscesses at first contained an opalescent, glairy, white fluid that subsequently became inspissated and opaque, and ultimately underwent calcification. Foreign body giant cells often were seen at the periphery of the calcified abscesses. An example is shown in Figure 1. As a rule, the abscesses about the elbows, shoulders, and knees were associated with proliferation of collagenous tissue about the joints, and periosteal new bone formation of the long bones near the affected joints. Usually, the joints were not invaded, but when they were, there was only slight, if any, erosion of the articular cartilage.

The bones of some of the animals that fared most poorly were quite fragile.

In three-fourths of the animals, necrosis of skeletal muscle was

present. This varied from necrosis of scattered fibers to involvement of the majority of the fibers in some muscles. Slight muscle injury was found in one animal after 18 days on the diet, but since extensive pneumonia, pericarditis, and empyema were present also, the possibility that muscle injury was due to infection must be considered. The earliest at which muscle necrosis was discovered in an animal with no signs of infection was after 58 days on the diet. Of interest is the fact that of the 35 animals that were kept on the diet more than 300 days, the muscles of 9 were normal, and in 8 animals only scattered fibers were necrotic; moderate to extensive necrosis was present in only 8 animals. Other lesions, to be described later, were seen in these 35 pigs.

Slightly injured muscles appeared grossly normal, but more severe injury was manifested by slight pallor and by the presence of opaque, grayish yellow, longitudinal streaks of various lengths and breadths. Occasionally, some of the streaks were coarse and beaded. In some animals these streaks were seen best or exclusively in the muscles of the chest wall, notably the serratus anterior and pectoral muscles, and the muscles attached to the scapula. In others they were clearly evident in the abdominal muscles and muscles of the legs also. As a matter of convenience and uniformity, sections were made routinely of the triceps brachialis and quadriceps femoris, and only occasionally of other muscles. We are consequently unable to state whether certain muscles had a greater susceptibility to injury than others.

Microscopically, there was necrosis with hyalinization, and in some cases with regeneration of muscle fibers. In some instances injury was manifested by slight to great increase in nuclei in some fibers, with necrosis of only an occasional fiber (Fig. 2). More commonly, however, the changes were predominantly degenerative and characterized by the presence of fragmented muscle fibers with swollen, hyaline, eosinophilic, curdy cytoplasm, and loss of nuclei (Fig. 3). Often an otherwise normal fiber was fractured and the terminal portion had the appearance just described. Some fibers had lost their nuclei, but the transverse striations were maintained or even accentuated. Still others showed loss of longitudinal striation. Some fibers were invaded by macrophages (Fig. 4), and in places these were aggregated to form foreign body giant cells associated with the fragmented cytoplasm (Fig. 5). Frequently, although muscles were the seat of much necrosis, few or no macrophages were present. In areas there was calcification, slight or fairly extensive, of the necrotic cytoplasm, and this was accompanied by macrophagic infiltration with or without giant cells

(Fig. 6). In a few instances the sole evidence of muscle injury was the presence of macrophages or giant cells in scattered fibers, with or without calcification. In the more severely injured muscles, entire fields consisted solely of necrotic fibers, and in the less injured ones, necrotic and viable fibers intermingled in various proportions. In the muscles that were described as containing beaded streaks there were nodules of more extensive calcification; it appeared probable that at least some of these had their origin in small abscesses. Slight fibrosis was associated with some of the last-mentioned lesions. In 2 animals the entire biceps brachii was calcified.

In fewer than 3 per cent of the muscles examined was there fatty infiltration, but in about one-half of those in which it occurred it was extensive (Fig. 7).

Sections of the median, radial, and femoral nerves of several animals showed no abnormalities. Sections were not stained for the demonstration of nerve endings, but in view of the findings of Rogers, Pappenheimer, and Goettsch[22] and of Chor and Dolkart[23] in vitamin E deficiency, it seems improbable that such stains would have shown anything.

The reason for muscle necrosis was not apparent. It is doubtful that it was due to vascular changes, for no blood vessel encountered in sections designed to show muscle had its lumen diminished in any manner, although calcification of the walls of small arteries was seen very rarely.

The mechanism of production of wrist stiffness was not always clear. In the animals with abscess formation and connective tissue proliferation about the joints it was obvious, but in those with only muscle necrosis it must have been functional.

In a few of the animals that had numerous calcified periarticular and intramuscular abscesses there was slight focal calcification of the subcutaneous tissue.

*Gastro-Intestinal Tract*

Calcification of the muscularis of the stomach or intestine, or both, was present in about one-sixth of the animals. In the stomach calcification was most common in the cardia and fundus near the greater curvature, and usually consisted of multiple, discrete, small deposits that rarely caused much thickening of the wall. In some animals an area of several square centimeters was involved. In the small intestine and colon the deposits were larger and caused appreciable thickening of the wall, but rarely involved a segment longer than 1.5 cm. In the

colon the deposits usually were located in a constantly sharply kinked loop situated a short distance from the cecum. Calcification was most common in the stomach and least common in the small intestine.

Microscopically, the deposits were seen to be of irregular size and shape. In a few instances, calcification of individual muscle cells was seen, but in general the masses appeared to have been deposited between the muscle cells, and frequently small groups of muscle cells extended about and between the deposits (Fig. 8). Associated with the deposits was a variable amount of collagenous tissue. Slight calcification in the muscularis mucosae was sometimes present, but there was no calcification of the mucosa or submucosa.

A lesion seen in several animals, but of doubtful relationship to the diet, was pneumatosis of the ascending colon and cecum. Gas bubbles of various sizes up to several millimeters in diameter were present in the submucosa and muscularis. The mucosa was intact.

*Liver and Gallbladder*

The gallbladder was often nearly empty, containing turbid bile, sometimes with minute pigment stones. In a few instances the bile seemed slightly purulent, and sections revealed moderately severe infiltration of the wall of the gallbladder by polymorphonuclear leukocytes, monocytes, and lymphocytes.

In about one-fourth of the animals the liver contained scattered, opaque, minute yellow dots or delicate yellow streaks, and in a few instances, subcapsular foci of necrosis a few millimeters in width. Microscopically, the dots and streaks were seen to be due to necrosis of liver cells. Rarely was there any leukocytic reaction, and when present it was slight. Often calcification of the necrotic cells was observed. Sometimes single cells or groups of only a few cells were calcified, but often the mass of calcified cells represented an appreciable fraction of the number of cells of the involved lobule. In the larger areas of necrosis involving several lobules it often happened that a narrow rim of viable cells persisted about the portal space, while many of the central and midzonal cells were calcified. In no instance was there enough injured tissue to indicate significant impairment of hepatic function.

*Kidneys*

The kidneys usually were pale, granular, somewhat enlarged, and heavier than is normal, both absolutely and in proportion to body weight. The granularity resulted from cortical scarring and hypertrophy of tubules between the scars. The scars varied in number and

size, and were characterized by the presence of atrophic convoluted tubules embedded in a small amount of collagenous tissue with a variable amount of lymphocytic infiltration, and glomeruli in the early stages of fibrosis. The glomeruli in these scars always seemed more nearly normal than the tubules. The appearance was somewhat suggestive of arteriolar nephrosclerosis in man, but differed in that the arterioles appeared normal. In badly scarred kidneys the intervening tubules were hypertrophic or dilated, or sometimes both hypertrophic and dilated; and in the less scarred ones many of the intervening tubules were normal. Calcified masses of irregular size and shape were seen in convoluted and collecting tubules, and at times foci of calcification of the interstitial tissue were seen in both cortex and medulla. Some of the masses obviously obstructed the tubules in which they lay. Calcification of the walls of arteries was seen in no more than 1 per cent of the kidneys examined, and in these the lumina were not reduced.

*Cardiovascular System*

The hearts of animals living more than 200 days on the diets usually were not sectioned, but the hearts of 60 per cent of those dying before 200 days were examined histologically. Of this group, about one-fourth contained foci of necrosis and calcification without inflammatory reaction in the ventricular myocardium (Fig. 9).

Lesions in the aorta or femoral arteries, or both, were found in about one-fifth of the animals dying before 150 days, in one-third of those dying after 150 days, and in nearly one-half of those dying after 300 or more days. Nodular lesions similar to those in the aorta sometimes were seen in the pulmonary artery. There was no constant relationship between the lesions in the aorta, pulmonary artery, and femoral arteries. In some cases all three were involved. The pulmonary artery was never affected alone, but the other two vessels were. Severe aortic lesions were not necessarily accompanied by lesions in the other vessels, and sometimes the aorta was normal while the femoral arteries were severely injured. The earliest aortic injury was observed after 113 days on the diet, and the earliest injury of a femoral artery was seen after 75 days on the diet.

Aortic injury was manifested grossly by the presence of small nodules in the intima distributed annularly, as is shown by Figure 10, and by bands of medial calcification with no appreciable thickening or with slight apparent thinning of the vessel wall. The intimal nodules, when not numerous, were seen only in the ascending aorta and aortic arch, but when more numerous were present in the thoracic aorta also. They

were rarely encountered in the abdominal aorta, but because of its small caliber, careful examination of this segment often was not feasible and the actual incidence of lesions here is not known. Microscopically, calcification of the media was found at times in association with foci of replacement of the elastica with collagenous tissue (Figs. 11 and 12), and at times without such replacement, calcification evidently having occurred in necrotic tissue without cellular reaction (Fig. 13). There were foci of destruction of elastic tissue, also, and replacement with rather cellular fibrous connective tissue without calcification. The underlying adventitia was usually normal, but occasionally showed some lymphocytic infiltration. The intimal nodules consisted of rather dense collagenous tissue and were invariably associated with scarring or calcification of the media (Fig. 12), but medial calcification was not always associated with intimal reaction, as is shown by Figure 13. In paraffin sections of the aorta there was no evidence that the intimal nodules contained cholesterol. Apparently the lesions began as medial necrosis with calcification, as shown in Figure 13, and subsequently or simultaneously collagenous tissue was formed in the larger lesions, giving the appearance seen in Figures 11 and 12. With the consequent weakening of the vessel wall there was a stimulus to formation of collagenous tissue in the intima.

The affected femoral arteries had a finely beaded appearance. Microscopic examination revealed fragmentation and reduplication, and often calcification of the internal elastic lamella; asymmetric intimal thickening, sometimes with focal calcification; and frequently, medial calcification (Figs. 14 to 16).

*Endocrine Glands*

In view of the manifest disturbance of calcium metabolism, the parathyroid glands were examined early, but since no abnormalities were found, this measure was abandoned. The adrenal glands were not remarkable.

*Controls*

Tissues from 54 guinea-pigs maintained upon the regular stock diet for periods of 170 to 675 days were available for comparison with those from animals upon deficient diets. In the skeletal muscles of 5 of these control animals a few scattered necrotic fibers were seen. The kidneys of 17 contained a few small cortical scars, and tubules of 3 contained a few small calcified masses. The livers of 4 controls contained one or a few foci of necrosis; 2 of these were visible grossly. It is thus apparent that although some of the lesions de-

scribed in the deficient animals were found also in the controls, their severity and incidence were much less.

### OBSERVATIONS ON HAMSTERS

It seemed of interest to determine whether hamsters would react as do guinea-pigs. Tissues of 13 hamsters were examined after the animals had received diet 2 for intervals of 108 to 777 days, but no lesions were found. Nine hamsters had been on the diet for more than 300 days, and 4 had been on it for more than 2 years.

## Discussion

Muscle injury resembling that encountered in our animals has been produced experimentally in a number of species, including guinea-pigs,[23-26] hamsters,[27] rats,[23,28-34] rabbits,[23-25,35] goats,[25] dogs,[36,37] mice,[38] tree kangaroos,[39] and ducklings,[40] by a variety of means, primarily by vitamin E deficiency, but also by an unidentified dietary deficiency,[24] administration of certain sulfonamides,[32-34] and feeding of dried eggwhite.[31]

Although the muscular changes in our guinea-pigs resembled those described by others, in view of the character and distribution of the other lesions we observed, it is apparent that the etiology of the injury resulting from administration of the skim milk diet differs from those previously reported. It must be admitted that the vitamin E intake may have been inadequate because of improper utilization or increased need, but this seems improbable in view of the absence of pigmentation of the intestines and of the failure of hamsters to develop similar lesions. Furthermore, vitamin E deficiency has not been found to produce vascular lesions like those we have shown.

## Summary

Administration of a diet prepared from dried skim milk powder supplemented with adequate amounts of the known vitamins leads to development of injury in the guinea-pig, but not in the hamster. The injury is characterized by development of a peculiar type of arteriosclerosis; necrosis and calcification of the skeletal muscle and myocardium; deposition of calcium salts in the smooth muscle of the gastro-intestinal tract, and in the kidneys and liver; and development adjacent to bones and joints of abscesses that frequently become calcified.

The animals examined in this study were cared for and observed clinically by C. A. Anderson and A. L. Caldwell, and were delivered to one of us (H) for necropsy at death or at the termination of an experiment.

## ADDENDUM

Since this article was submitted for publication, a paper that may have some bearing upon the vascular lesions has come to our attention. This was by White, J., and Mider, G. B., The effect of dietary cystine on the reaction of dilute brown mice to methylcholanthrene (preliminary report). *J. Nat. Cancer Inst.*, 1941–42, **2**, 95–97. Sclerotic lesions of the aorta and large arteries occurred frequently among mice that were painted repeatedly with a solution of methylcholanthrene and that were given a diet low in cystine. These lesions bore a resemblance to those we have described.

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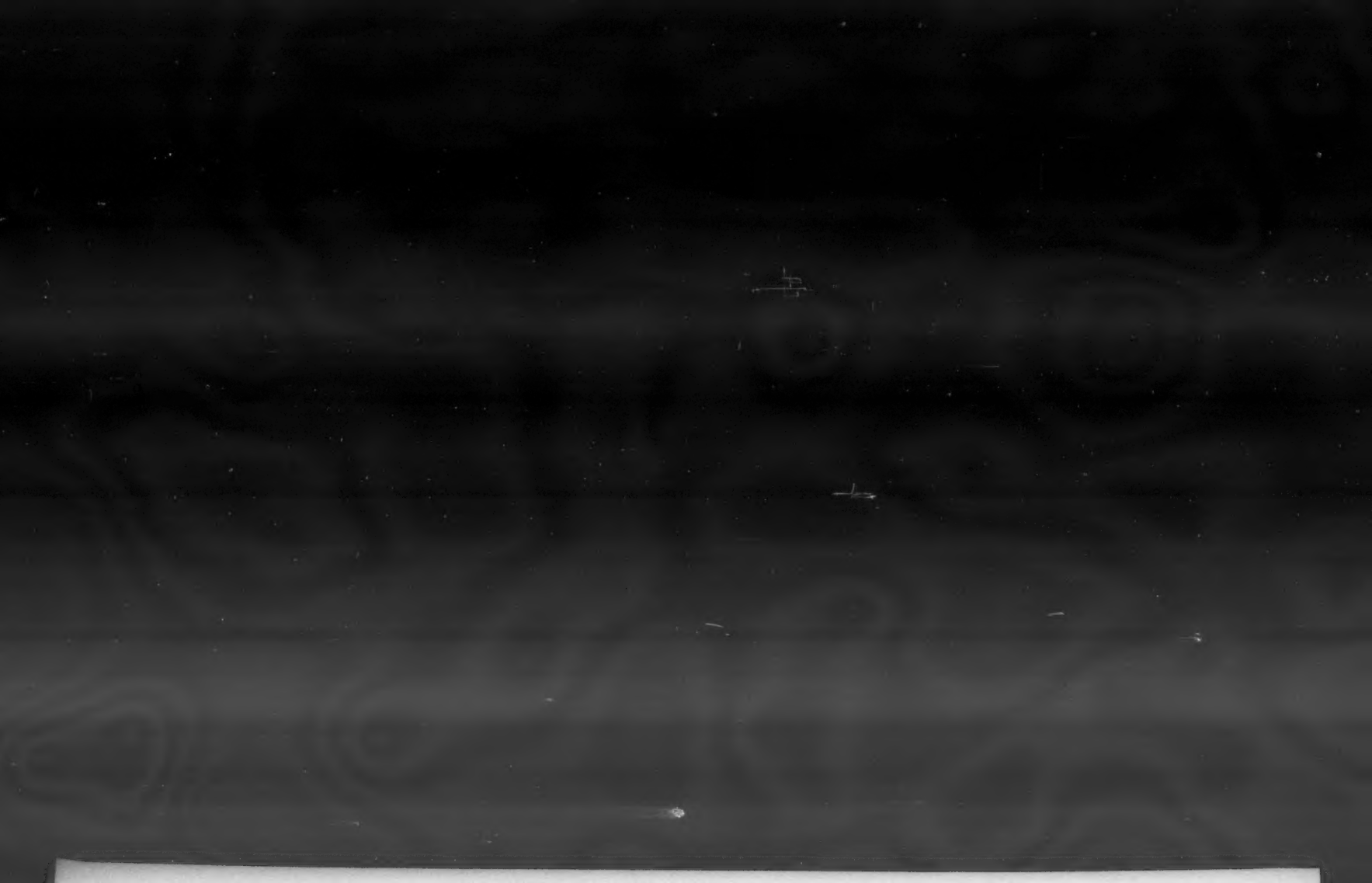
## DESCRIPTION OF PLATES

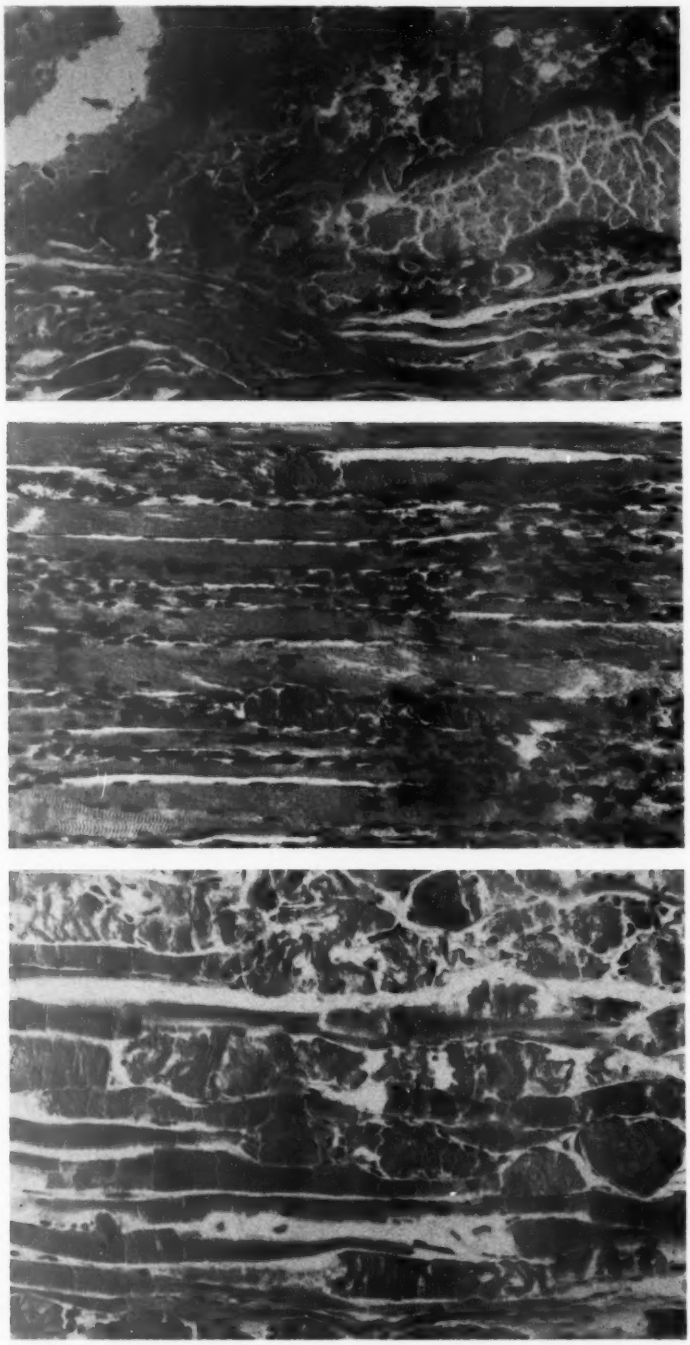
### PLATE 87

FIG. 1. Skeletal muscle. Diet 2, 100 days. There is a multilocular abscess with calcification, peripheral macrophagic reaction, and foreign body giant cell formation. × 190.

FIG. 2. Skeletal muscle. Diet 2, 140 days. There is great increase in number of nuclei of muscle fibers and perhaps of sarcolemma also. A few necrotic muscle fibers are included. × 190.

FIG. 3. Skeletal muscle. Diet 2, 156 days. Many muscle fibers are fragmented, swollen, curdy, and anuclear. A few atrophic and regenerating fibers are present. × 190.






Harris and Wulzen Diets Deficient in Anti-Stiffness Factor

PLATE 88

FIG. 4. Skeletal muscle. Diet 2, 439 days. This differs from Figure 3 in that there is much macrophagic infiltration of necrotic fibers. × 190.

FIG. 5. Skeletal muscle. Diet 2, 289 days. There is much necrosis with macrophagic infiltration, foreign body giant cell formation, and calcification of some necrotic tissue. × 190.

FIG. 6. Skeletal muscle. Diet 2, 149 days. There is much calcification of the necrotic tissue with moderate macrophagic infiltration. × 190.

4

5

6

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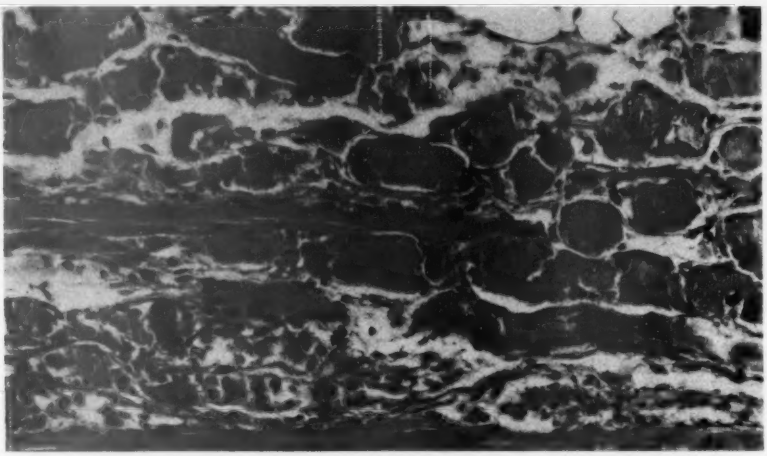

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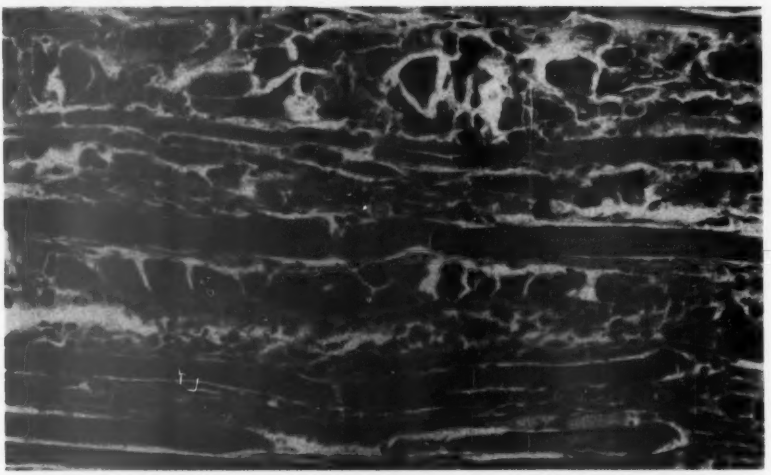

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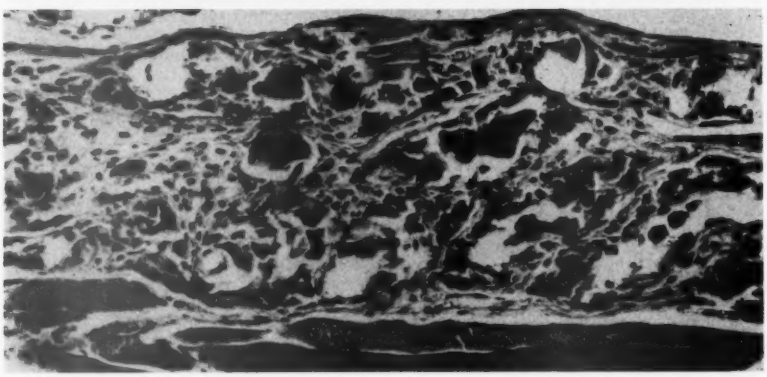


Harris and Wulzen Diets Deficient in Anti-Stiffness Factor

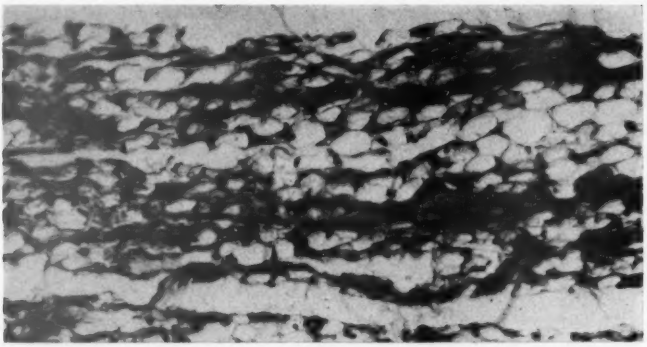
PLATE 89

FIG. 7. Skeletal muscle. Diet 1, 374 days. There is extreme atrophy of muscle with much fatty infiltration. × 190.

FIG. 8. Colon. Diet 1, 210 days. The muscularis contains irregular calcified masses surrounded by rather loose collagenous tissue and small numbers of macrophages. Bundles of attenuated smooth muscle cells are seen at the left and at the top and bottom. × 190.

FIG. 9. Heart. Diet 2, 87 days. There are several foci of calcification of myocardial fibers. × 190.

7



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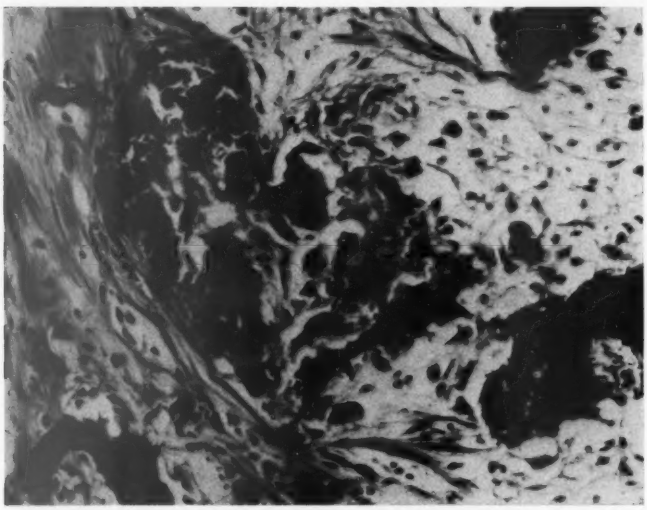

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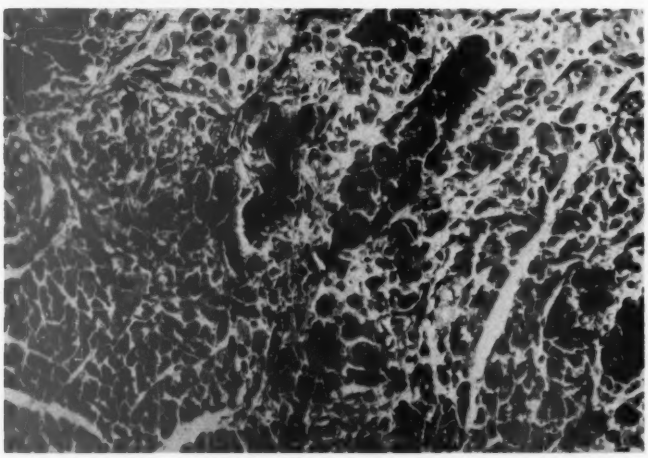


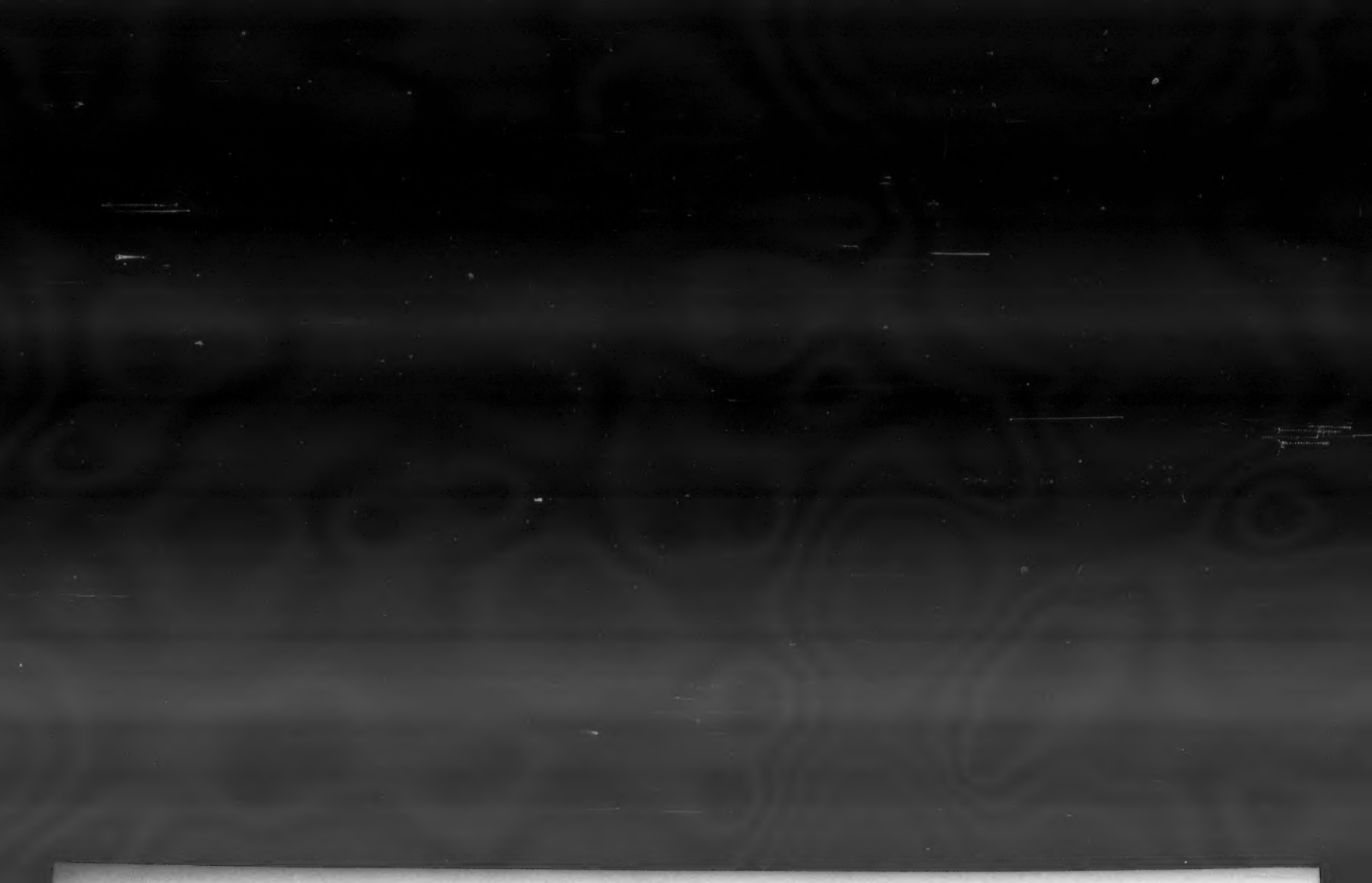
Harris and Wulzen Diets Deficient in Anti-Stiffness Factor

PLATE 90

FIG. 10. Aorta. Diet 2, 329 days. There are transverse rows of intimal plaques. The distal portion, at the right, is the seat of medial calcification without intimal change. × 3.

FIG. 11. Aorta. Diet 23, 131 days. There is a large region of medial calcification with slight scarring and slight intimal thickening. × 190.

FIG. 12. Aorta. Diet 2, 371 days. There is a medial scar with loss of elastic tissue, replacement with collagenous tissue, slight cellular infiltration, some calcification, and thickening of the overlying intima by a plaque of collagenous tissue. × 190.



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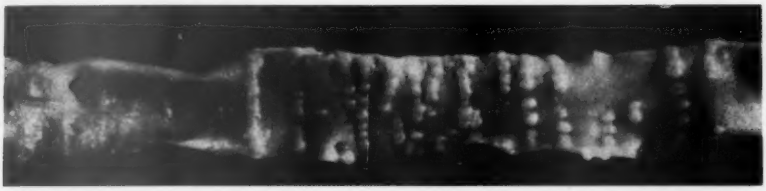

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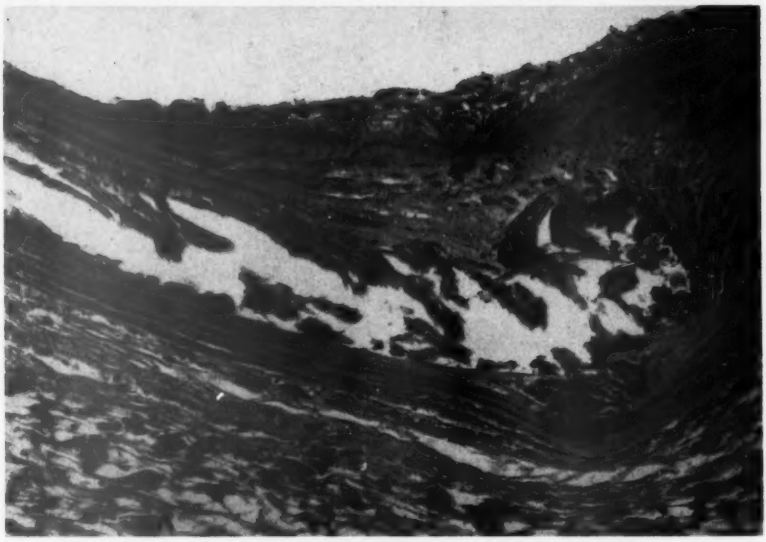

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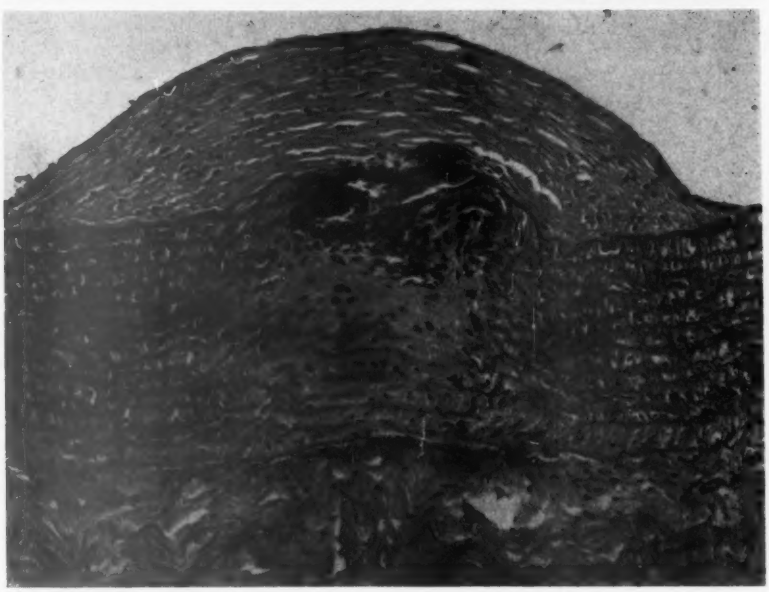

Harris and Wulzen Diets Deficient in Anti-Stiffness Factor

PLATE 91

FIG. 13. Aorta. Diet 2, 152 days. There is a focus of medial calcification with no change in the overlying intima. × 190.

FIG. 14. Femoral artery. Diet 2, 308 days. There is much intimal thickening. Only a small segment of the internal elastic lamella persists, and the underlying media is scarred. Elsewhere the media shows much calcification. × 190.

FIG. 15. Branch of the femoral artery. Diet 2, 430 days. There is much asymmetric intimal thickening with fragmentation and calcification of the internal elastic lamella. Some calcium deposits are large, apparently involve both media and intima, and are associated with some macrophagic reaction. × 190.

FIG. 16. Branch of the femoral artery. Diet 2, 131 days. The intima is greatly thickened, and the internal elastic lamella is calcified and fragmented. Some foci of calcification about this membrane involve both intima and media. × 190.

13

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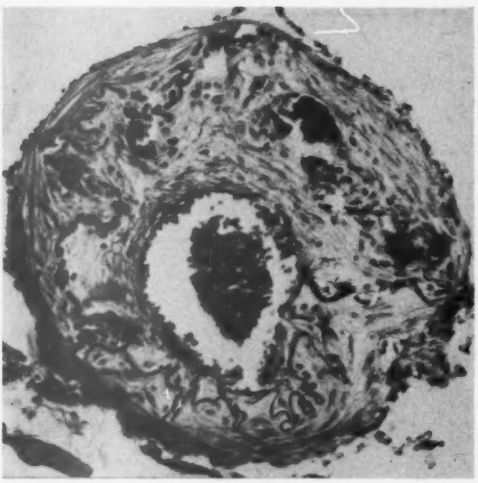
15



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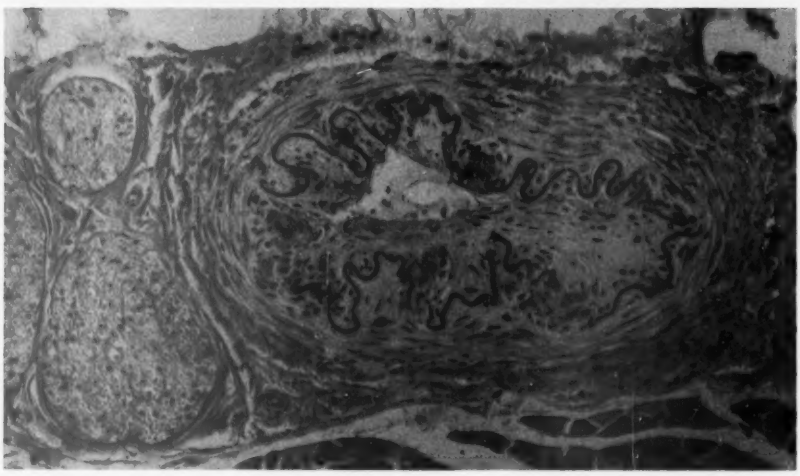

Harris and Wulzen Diets Deficient in Anti-Stiffness Factor

# THE PATHOLOGY AND ETIOLOGY OF SALMON DISEASE IN THE DOG AND FOX*

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So-called salmon "poisoning" has been recognized for many years in the Pacific Northwest, where the disease occurs west of the Cascade Mountains from northwestern California to the vicinity of Olympia, Washington. Dogs are the common natural host, although the disease was found in ranch-raised foxes by Donham, Simms, and Miller[1] and the coyote has been infected experimentally by Donham and Simms.[2]

While the specific causative agent has not been demonstrated previously, evidence has accumulated indicating that the natural vector is a small intestinal fluke, *Troglotrema salmincola*. Encysted metacercariae in fish of the family Salmonidae are regarded as the means of infecting the carnivore. Ova passed by adult flukes in the carnivore intestine develop miracidia which infect the snail, *Goniobasis plicifera* var. *silicula*. It appears that the range of this specific snail limits the area of occurrence of the disease in carnivores. Cercariae escape from the snail and infect suitable species of fish to complete the life cycle of the fluke.

Experimentally, Simms, McCapes, and Muth,[3] and Simms and Muth[4] have transmitted the infection by intraperitoneal injection of blood or ground, washed flukes from infected dogs, and metacercariae from fish. Kennel contact and the feeding of intestinal contents, flukes, or blood from infected dogs were without effect. Intraperitoneal injection of rediae and cercariae from snails, of blood from fluke-infected salmon, and of Mandler (medium) and Seitz filtrates of blood or ground flukes from infected dogs all failed to induce the disease.

As earlier workers dealt chiefly with transmission and immunization, it was thought essential to make a more thorough study of the gross and microscopic lesions of the disease. Early in the work the elementary bodies were discovered and investigation of both pathogenesis and etiology was undertaken.

## Materials and Methods

The foxes used in these studies were Silver and Silver-Whiteface animals of both sexes grown on the Fur Animal Disease Research unit. Most of the animals were about 1 year of age, but a few were older. They were housed in individual cages and observed daily.

* Received for publication, July 15, 1949.

The dogs used were of mixed breeding and both sexes obtained locally in eastern Washington outside the area of the natural disease. Dogs 1 to 8 inclusive were 4 months of age. The others were about 1 to 3 years old. Dogs 1 to 9 inclusive received one or two doses of canine distemper antiserum subcutaneously. All dogs were observed twice daily and afternoon temperatures recorded. Both foxes and dogs were killed by ether inhalation and exsanguination.

The two cats used were adult males. The mink were adults of standard type raised on the Fur Animal Disease Research unit. The guinea-pigs and hamsters were mostly half-grown animals from the stock colony.

Webster Swiss white mice raised in isolation were used for passage work. Mice of both sexes, 3 to 5 weeks old, were employed. The colony had twice been passaged intranasally through 5 to 7 passages without the appearance of latent virus infections.

The eggs used for inoculation were from commercial hatchery stocks of White Leghorn and New Hampshire Red chickens. They were incubated 6 days at the hatchery before inoculation.

The three lots of fish used in feeding experiments were hatchery cutthroat trout furnished through the courtesy of Dr. J. N. Shaw of Oregon State College. The specimens were all found to contain large numbers of fluke metacercariae.

Tissue for inoculation from the dog or fox was removed aseptically at necropsy and placed in a sterile, weighed, semimicrocontainer for the Waring blender, where it was diluted to make a suspension with sterile skim milk, broth, or saline solution.

Bacteriologic examination of dog or fox organs (lymph nodes, spleen, and occasionally liver) or of suspensions for injection was usually accomplished by inoculation of blood agar plates. In a number of cases thioglycolate broth and Sabouraud's maltose agar were employed also. A number of animals were examined for enteric bacterial pathogens by inoculation of Kaufmann's brilliant green agar after preliminary enrichment in tetrathionate broth.

Tissues taken at necropsy were fixed in 10 per cent formalin and routinely stained with hematoxylin and eosin, and by Giemsa's method. The Gram, Pollak, and Levaditi methods were used on certain tissues. Smears were stained by Giemsa's and Macchiavello's methods.

*Experiment I.* In November, 1947, Dr. H. J. Griffiths fed several ounces of infected fish (lot I) to 2 dogs (D-01 and D-02) to demonstrate the disease to the class in veterinary parasitology. At the same time one of us (J.R.G.) fed 15 oz. of the same fish to each of 3 foxes (F-01, F-02, and F-03) to observe the disease in that species. Typical symptoms

appeared in all 5 animals. All were allowed to die and necropsies were performed within 1 to 12 hours. Low environmental temperatures minimized autolytic changes. It was the histopathologic examination of these animals which led to the discovery of the elementary bodies.

*Experiment II.* On March 3, 1949, 6 gm. of fluke-infected fish (lot II) was fed to each of 3 dogs (D-1, D-2, and D-3) and 28.5 gm. to each of 2 foxes (F-1 and F-2). All remained normal for 2 months when they were challenged by intraperitoneal injection (experiment V). Fluke ova were demonstrated in fecal specimens of all 5 animals.

*Experiment III.* On April 22, 1949, 30 gm. of fish (lot III) was fed to each of 2 foxes (F-4 and F-5) and 12 gm. to each of 2 dogs (D-4 and D-5). F-5, D-4, and D-5 remained healthy for 38 days when they were challenged by intraperitoneal injection (experiment VII). F-4 showed typical symptoms and was destroyed on the 15th day. Flukes were present at necropsy in duodenal scrapings of F-4. Fluke ova were found also in fecal specimens from the other 3 animals.

*Experiment IV.* On May 7, 1949, a 25 per cent suspension of spleen and abdominal lymph nodes from F-4 was prepared in sterile skim milk. Two foxes (F-6 and F-7) each received 2.5 cc. intraperitoneally and 2 dogs (D-6 and D-7) were each given 2 cc. intraperitoneally. One mink was given 1.25 cc. by the same route. All of the foxes and dogs developed the disease in typical form and were sacrificed. The mink remained healthy for 6 weeks, when observations ceased.

*Experiment V.* At the time of experiment IV and using the same inoculum, 2 cc. was given intraperitoneally to each of 3 dogs (D-1, D-2, and D-3) and 2.5 cc. to each of 2 foxes (F-1 and F-2). These animals had all failed to show illness after feeding fish of lot II (experiment II) 65 days earlier. All became typically ill following intraperitoneal challenge. The 2 foxes died under observation and necropsy followed immediately. The dogs were all destroyed at the height of the disease. Flukes still were present in the duodenum of D-1 and F-1, but unfortunately the other specimens were lost.

*Experiment VI.* On May 16, 1949, one dog (D-8) and one fox (F-8) were each given 2 cc. intraperitoneally of a 10 per cent broth suspension of spleen and lymph nodes from D-6 (experiment IV). Both animals showed typical symptoms. The fox died on the 11th day and the dog was destroyed on the 14th day.

*Experiment VII.* On May 30, 1949, D-8 (experiment VI) was destroyed and a 10 per cent broth suspension of spleen and lymph nodes prepared. Two foxes (F-5 and F-10) were each given 4 cc. intraperitoneally and 2 dogs (D-4 and D-5) each received 2 cc. by the same route.

F-5, D-4, and D-5 had previously shown no illness when fed fish of lot III (experiment III) 38 days earlier. All 4 animals developed typical symptoms. The foxes were allowed to die; the dogs were killed.

*Experiment VIII.* On May 18, 1949, F-7 (experiment IV) was destroyed and a 10 per cent broth suspension of lymph nodes prepared. This inoculum was given intraperitoneally to fox F-9 (3 cc.), 2 cats (2 cc.), 3 mink (1.25 cc.), 7 guinea-pigs (0.5 cc.), and 10 hamsters (0.5 cc.). No symptoms were shown by any of the animals except the fox, which became typically ill and was killed on the 16th day.

Using the same inoculum, mouse passages were begun. Groups of 5 to 7 mice, 3 to 5 weeks old, were used. Three blind serial passages were made by each of three routes. Intraperitoneally, 0.4 cc. was used, employing fox lymph node suspension in the first passage and 10 per cent broth suspension of pooled mouse spleens in the second and third passages. Intranasally, 0.05 cc. was given, using pooled mouse lungs in second and third passages. Intracerebrally 0.03 cc. was employed, using pooled mouse brains for the later passages. At no time were gross lesions apparent in any mice.

Using the same fox lymph node suspension, 4 dozen eggs incubated 6 days were inoculated into the yolk sac with the original 1:10 dilution and with 1:100, 1:1000, and 1:5000 dilutions in 1 cc. amounts. Following 6 days of further incubation at 37° C., the yolk sacs were harvested. A number of yolk sacs of the 1:10 and 1:100 dilutions were pooled and a 10 per cent broth suspension prepared. This was used to inoculate a further passage of eggs in 1:10, 1:100, and 1:1000 dilutions. No elementary bodies were seen in any smears of inoculated or control eggs of either passage.

*Experiment IX.* On June 1, 1949, a 50 per cent saline suspension of pooled drained yolk sacs from 5 second-passage 1:100 dilution eggs (experiment VIII) was prepared. One dog (D-9) and one fox (F-11) each received 4 cc. intraperitoneally. Neither animal showed any illness for 17 days, when both were challenged (experiment XI).

*Experiment X.* On June 2, 1949, the organs were harvested from the third passage of mice (experiment VIII). That is, the brains were collected from intracerebrally passaged, the lungs from intranasally passaged, and the spleens from intraperitoneally passaged mice. All of these organs were pooled and a 17 per cent saline suspension prepared. One fox (F-12) was given 8 cc. intraperitoneally and one dog (D-10) received 6 cc. by the same route. Both animals developed typical symptoms. The dog was killed and the fox allowed to die.

*Experiment XI.* On June 18, 1949, a 20 per cent broth suspension of lymph nodes from dog D-10 (experiment X) was prepared. The nodes had been held 4 days in the refrigerator. Two dogs (D-9 and D-11) and a fox (F-11) were each given 2 cc. intraperitoneally. D-9 and F-11 had shown no illness after being given egg yolk sac material 17 days earlier (experiment IX). All 3 animals developed typical symptoms and were killed on the 11th day.

## Results

In all, 4 foxes and 2 dogs were infected by feeding fish and 10 foxes and 11 dogs were infected intraperitoneally by serial passage of tissue suspensions from F-4. Nine foxes and 2 dogs were allowed to die, succumbing at 11 to 15 days (average, 13.5 days) after feeding or injection. Five foxes and 11 dogs were destroyed at 9 to 16 days after exposure (average, 11.8 days).

### *Symptomatology*

Dogs infected by feeding fish showed a rise in temperature to over 104° F. on the fifth day. Dogs infected intraperitoneally usually showed the first hyperthermia on the fourth day, although in a few cases it occurred on the third, fifth, or sixth days. Temperatures of 104° to 106° F. were continuous for 4 to 8 days, when the dog was either destroyed or the temperature dropped to normal or below. Temperatures were not taken in foxes because of the irregular rises induced by handling.

Coincident with the onset of fever, or a day or two later, there was anorexia. This was the first symptom observed in foxes and usually occurred at 5 to 7 days. Usually anorexia was very marked and often complete, especially in foxes. The route of infection had no influence on this symptom. The animals continued to take little or no food during the entire course of the disease. After a few days of illness, a marked weight loss became apparent. At the same time, the animals showed deep depression and weakness. If allowed to live, diarrhea and often vomition appeared. The feces usually were scanty, yellowish, and mucoid or watery. Defecation was accompanied by tenesmus in many cases. Some blood was seen in feces passed by animals infected by feeding, but was infrequent in those infected parenterally. Blood appeared in the feces of only 3 of 11 dogs infected intraperitoneally, although 10 showed diarrhea.

Early in the febrile period, occasional serous nasal discharge was seen. In many animals which had shown fever for several days, a gummy conjunctival exudate was seen at the inner canthus.

Symptoms in these experimental foxes and dogs were very similar to

those reported by others. Donham[5] found an incubation period of 6 or 7 days in dogs fed infected fish, but reported occasional animals with a period of 5 to 12 days of incubation. Generally this is somewhat longer than seen in our dogs, most of which received large inocula intraperitoneally. There appears to be no reason for suspecting that we have been dealing with a disease other than typical salmon "poisoning."

### *Gross Pathology*

Macroscopic lesions were consistent in both dogs and foxes, but were more severe in foxes. This may have been because the foxes were given larger inocula and often were allowed to die after a longer course, but it appears likely that the fox is actually more susceptible than the dog. Changes in the lymphoid tissues appeared to be primary and were found in all cases in varying degree.

Both visceral and somatic lymph nodes were affected. Infection by feeding or by intraperitoneal injection did not appear to affect markedly the extent or location of lymph node involvement. Variable enlargement of most of the nodes was typical. The swelling was most extreme in the abdominal nodes (ileocecal, colic, mesenteric, portal, and lumbar). In some cases the ileocecal and mesenteric nodes were enlarged three to six fold. The lumbar nodes also were often markedly swollen. In some cases there was moderate enlargement of the pharyngeal, pre-scapular, bronchial, mandibular, mediastinal, or external inguinal nodes. Usually the nodes were yellowish with prominent white foci representing the cortical follicles. Occasional nodes showed small hemorrhages or diffuse redness. Edema was often observed about some nodes. Some of the extremely swollen nodes showed softening and an opaque, grayish fluid could be expressed from them.

The tonsils often were enlarged and everted from the fossae. Usually they were yellowish with the follicles appearing as prominent white foci. Occasionally the tonsils showed petechiae or were diffusely pink.

The spleen often showed some enlargement, varying from slightly greater plumpness to twice the normal volume. The splenic follicles usually were apparent as grayish white nodules in fox spleens, but often were unrecognizable in dogs. In animals that had been killed, the spleen had a normal plum-red color, slightly irregular surface, and plastic firmness. Animals allowed to die showed spleens which were a darker bluish red, smooth, somewhat softer, and more blood-filled.

The lymphoid tissue of the intestine usually was prominently enlarged. The aggregated lymph follicles of the small intestine (Fig. 1) were es-

pecially swollen and yellowish white. The solitary follicles of the pylorus and large intestine were extensively affected also.

The intestinal contents showed a large amount of blood in 4 of 6 animals infected by feeding. Among the animals infected by intraperitoneal injection, free blood in the contents was infrequent. Two foxes of this group that died had a large amount of blood in the lumen of the anterior small intestine. Three foxes and one dog showed a small amount of free blood in the colonic and rectal contents. Usually the intestine was empty except for a small amount of bile-stained mucus.

Many of the injected animals showed petechiae in the gastro-intestinal mucosa. One had petechiae in the lower esophagus. Five foxes and 9 dogs showed petechiae in the pyloric mucosa. Three foxes and 2 dogs had small hemorrhages in the mucosa of the small intestine. Six foxes and 9 dogs had petechiae in the large intestine, especially in the ileocolic valve, posterior colon, and rectum. Often these small hemorrhages appeared to occur over enlarged lymph follicles. One fox showed several small bleeding ulcers in the pylorus. Three dogs had petechiae in the gastric serosa. One dog, infected by feeding, showed a 7 cm. intussusception of the ileum into the colon as a terminal condition. Donham[5] reported 5 intussusceptions among 74 cases produced by feeding fish.

In most of the dogs the liver appeared normal. In most of the foxes it was soft, friable, and pale yellowish brown. Two foxes showed hemorrhages in the gallbladder wall. Four injected foxes showed extensive hemoperitoneum. Three of these animals had ruptured livers. Three animals had died and one was killed in extremis.

A few petechiae were observed under the serosa of the pancreas in one dog.

Grossly, the kidneys were normal in the dogs. The kidneys of the foxes had pale yellowish brown cortices.

The mucosa of the urinary bladder showed small irregular hemorrhages up to 3 mm. in diameter in 3 foxes infected by feeding and in 9 foxes and 6 dogs infected parenterally. In no case was the urine blood-tinged, being yellow and clear or slightly turbid in all.

No lesions were observed in the heart. Most of the foxes showed raised, white foci of calcification in the aortic wall just above the valves. The foci were 1 or 2 mm. in diameter. These lesions obviously antedated the current infection and are presumed to be unrelated.

Pulmonary hemorrhage (Fig. 2) was prominent in 7 animals, including 3 foxes and a dog infected by feeding and 3 foxes infected parenterally. All had died except one fox which had been killed when mori-

bund. The lungs were studded with bright red or dark red, round, hemorrhagic areas, 5 to 20 mm. in diameter. A few scattered pulmonary petechiae were observed in 4 other animals. It would appear that pulmonary hemorrhage is related to the terminal stages of the disease, since 9 of 11 examples were found in animals that died. Several lungs showed tiny, gray or dull pink, impalpable foci scattered under the pleura in otherwise normal tissue.

The thymus was often yellowish white, enlarged, and soft. A few petechiae were observed in 6 foxes and one dog. Edema in the anterior mediastinum was seen in several animals.

Marked icterus occurred in 2 foxes (F-1 and F-2) infected parenterally. A slight yellowish discoloration was observed in a few other foxes.

Subcutaneous ecchymoses over the back and sides were seen in one dog.

No other lesions were observed macroscopically. Except for the lumbar spinal cord, which was normal in 2 animals, the central nervous system was not examined.

### *Histopathology*

Microscopically, similar lesions were found in all lymphoid tissues. These changes were generally much more severe in foxes. The lymph nodes showed a marked and consistent depletion of small lymphocytes with hyperplasia of reticulo-endothelial cells in the cortex and medulla. In most foxes and occasional dogs there were large foci of necrosis in the cortical tissue (Fig. 3) with small scattered foci in the medullary cords. Neutrophils were numerous in and about these foci, as well as scattered diffusely throughout the parenchyma and sinuses. Large macrophages were increased in the sinuses. Often there were small hemorrhages in the necrotic foci or erythrocytes in the sinuses. With Levaditi staining it was noted that some disruption of the reticulum occurred in the necrotic foci in the cortex. Elementary bodies were observed in reticulo-endothelial cells in the sinuses and in the parenchyma. In most of the dogs and in occasional foxes the lesions were much less severe. Only scattered neutrophils and occasional tiny necrotic foci or hemorrhages were present. Macrophages filled with elementary bodies were fewer. The lymph follicles of the intestine were similarly affected and showed the same difference in severity of involvement as between foxes and dogs. The aggregated follicles of the small intestine and the solitary follicles of the large intestine were about equally affected in any one animal. Solitary follicles in the pyloric mucosa were similarly

damaged and had ulcerated in one fox. The tonsils showed the same changes, but were generally less severely involved, even in foxes.

The spleen (Fig. 4) was affected in the same manner as the other lymphoid tissues. The splenic follicles usually showed much less necrosis and more frequent central hemorrhages. Neutrophils were present in variable numbers in both white and red pulp. Elementary bodies were found in macrophages in the splenic follicles and in the pulp cords and sinuses.

The thymus in younger dogs and foxes often was severely affected. There was marked depletion of small lymphocytes (thymocytes). There remained large reticular cells and macrophages which contained many elementary bodies. Neutrophils were numerous. A few tiny foci of necrosis were observed. Edema was seen in some cases.

Sections of stomach were normal except for follicular damage and occasional small hemorrhages into the lamina propria of the pyloric mucosa.

Other intestinal lesions were consistently present in addition to the lesions of lymph follicles. In animals to which fish had been fed, flukes (Fig. 5) were found embedded among the villi or in the duodenal glands. The parasites were not accompanied by any notable necrosis or leukocytic infiltration. In most animals, whether infected by feeding or by intraperitoneal injection, there were tiny foci of macrophages and neutrophils, frequently with necrosis, in the connective tissue of the lamina propria. These were especially common in the villi, but occurred also among the intestinal glands. The diffuse scattering of leukocytes in the lamina propria appeared to be little greater than is normal. Most of the cells were mononuclears, especially plasma cells, with some neutrophils and eosinophils. Small hemorrhages were seen occasionally in the lamina propria, especially in the colon and rectum. There was scattered loss of surface epithelium, especially over the lymph follicles. In some animals, necrosis of the distal ends of the villi occurred in the duodenum. Lesions were somewhat more severe in those infected by feeding and in those carrying a fluke infection at the time of intraperitoneal inoculation. The mucosa was affected similarly throughout both the small and large intestines. Except for a small amount of mononuclear infiltration around severely injured lymph follicles and some edema in the colon, the submucosa was unaffected. The muscularis and serosa were normal.

In foxes the liver showed extensive centrolobular lipidosis. This was absent or slight in dogs. This may have been largely due to fasting, as

the foxes showed more complete anorexia and were allowed to live, on the average, 2 days longer than the dogs. Occasional necrotic cells were seen about the central vein in foxes. Most of the fox livers showed bile stasis and accumulations of greenish yellow pigment granules in the hepatic and Kupffer cells. A moderate and variable amount of mononuclear infiltration in the interlobular connective tissue was seen in both foxes and dogs. Two foxes showed hemorrhages in the gallbladder wall.

In the dogs there were no renal lesions. Most of the foxes showed lipid droplets in the tubular epithelium, especially in the renal rays. Since 2 normal foxes showed the same picture, it is believed that the fox, like the cat, normally shows this phenomenon.

Sections of urinary bladder from both dogs and foxes often showed small hemorrhages in the subepithelial connective tissue. There was no accompanying leukocytic infiltration, and the surface epithelium was intact.

The lesions seen in the base of the aorta in most of the foxes were foci of calcium deposition in both intima and media. The foci often involved nearly the entire thickness of the wall. Some hyalinization was observed about the deposits, but no leukocytic infiltration.

Sections of lung showed small patchy areas in which the alveolar walls were thickened by accumulations of mononuclears and some neutrophils. No appreciable alveolar or bronchial exudate was present. In some lungs there were large, irregular areas of alveolar hemorrhage.

Two foxes showed small hemorrhages in the adrenal cortex.

A mononuclear infiltration of periductal connective tissues around the larger ducts of the pancreas was observed in several animals.

The thyroid gland was normal.

### *Hematology*

Systematic blood studies were not conducted. Examinations were made on 4 dogs (D-4, D-5, D-9, and D-10) killed at the height of the disease on the 11th or 12th day. Erythrocyte counts were 7.0, 7.5, 5.0, and 8.5 millions per cmm., respectively. These are normal or high normal counts and perhaps indicate some hemoconcentration due to vomition or diarrhea. The leukocyte counts were somewhat elevated: D-4, 18,500 per cmm.; D-5, 14,700; D-9, 22,000; and D-10, 28,000. Differential counts showed 90 to 93 per cent neutrophils, including many immature forms. The remaining cells were monocytes and lymphocytes in about equal numbers. No eosinophils were observed in counting 200 cells for each dog and in further examination of the smears. A neutrophilic

leukocytosis would appear to characterize the disease, judging from such scanty information. An absolute lymphopenia may occur also.

### Field Case in a Fox

On May 26, 1949, the carcass of an adult Platinum fox was received from a ranch at Hood River, Oregon. On gross examination there was observed the same enlargement of lymphoid organs as seen in experimental foxes. Small hemorrhages were found in the mucosa of the urinary bladder, although none were present in the lungs. The liver and kidneys were pale and soft. Flukes were found in duodenal scrapings. Microscopically, the spleen, lymph nodes, and tonsils were affected typically. Many elementary bodies were seen in lymph node smears and in sections of lymphoid organs.

The owner had fed smelt 60 days earlier, but reported no feeding of fish subsequently. About a month after feeding the smelt, he lost 3 dogs which had been ranging freely. Since then he had lost 7 or 8 foxes. While the dogs might easily have obtained infected fish, it is difficult to account for the transmission of the disease to the caged foxes.*

### Discussion

The gross and microscopic lesions found in our experimental animals are not in complete agreement with those found by earlier workers. Donham[5] reported finding a marked hemorrhagic inflammation from pylorus to anus in 74 dogs affected by feeding fish. Usually there was free blood in the lumen. Enteritis was present in areas where no flukes were embedded. Simms, Donham, and Shaw[6] found that the ileocolic valve, rectum, and posterior ileum usually were affected most seriously. Donham[5] and Hoeppli[7] reported loss of surface epithelium and necrosis in the subepithelial portion of the lamina propria. Our animals showed a moderate enteritis characterized by focal necrosis, leukocytic infiltration, and some hemorrhage. The presence of flukes did not increase significantly the severity of the inflammation. Most of the small amount of blood seen in feces or in colonic contents appeared to come from small hemorrhages associated with the solitary follicles in the colon and rectum.

Donham[5] reported marked swelling of the ileocecal lymph nodes in many of his dogs. Some of these nodes were purulent. A few animals showed moderate swelling of the mesenteric nodes. Simms, Donham, and Shaw[6] saw some swelling in cervical nodes. Lymph node enlarge-

* Eventually 25 of 75 foxes died. None of the mink on the ranch were sick. Apparently the source of infection was trout trimmings added to the ration which was fed to dogs, foxes, and mink alike.

ment was very constant in our animals. Involvement of the tonsils, thymus, and spleen has not been reported by earlier workers.

Hemorrhages in the urinary bladder, lungs, and thymus were not observed previously. This may have been because earlier investigators used dogs, in which such lesions are much less frequent than in foxes. The same is true of the fatty changes in the liver and kidneys.

The interstitial pneumonia was never extensive and was not evident clinically.

*Etiology*

In all of the foxes and dogs, whether infected by feeding or by intraperitoneal injection, small intracytoplasmic bodies (Figs. 6, 7, and 8) were observed in the large reticulo-endothelial cells of lymph nodes, tonsils, spleen, and intestinal lymph follicles. The bodies also were very numerous in the thymus. They were seen occasionally in macrophages of the liver, lungs, and blood. In some parenterally infected animals they were found in serosal macrophages. These bodies were coccoid or coccobacillary, and of a uniform size of about 300 m$\mu$. They stained purple with Giemsa's stain, red or blue with Macchiavello's, pale violet with Pollak's, pale bluish violet with hematoxylin and eosin, black or dark brown with Levaditi's stain, and were Gram-negative. Giemsa's stain was very satisfactory for routine demonstration in smears or sections. The bodies were found in the cytoplasm of reticulo-endothelial cells in compact plaques or loose groups, often nearly filling the cytoplasm. In severely damaged areas they were found free, as though released by cell disintegration. In such areas they were observed occasionally in neutrophils. The bodies were never observed in epithelium, endothelium, fibroblasts, or muscle cells. In many preparations, bodies suggesting the larger initial bodies of the lymphogranuloma-psittacosis group were seen. A matrix of glycogen could not be demonstrated about these bodies by either carmine or iodine staining.

Identical bodies were observed in cases infected from three different sources: 5 animals infected by feeding fish of lot I in 1947; 10 foxes and 11 dogs infected by various intraperitoneal passages of material from a fox infected by feeding fish of lot III in 1949; a fox from the field outbreak on the Oregon fur ranch. No bodies of either type were found in 2 normal foxes and 2 normal dogs. It is believed that the bodies are the etiologic agent of the disease.

Bacteria could not be implicated as a cause of the disease. Blood agar plates inoculated from spleen and lymph nodes showed no growth in 4 foxes and 9 dogs. Various cocci and rods grew on one or more plates from 4 foxes and 2 dogs. Three of these foxes had been allowed

to die. Only a few colonies appeared on media inoculated from the 2 dogs. No additional organisms were isolated by use of thioglycolate broth except for Gram-positive rods in one of the dogs mentioned above. It is presumed that these occasional organisms represent only contamination or late secondary bacterial infection. No growth appeared on Sabouraud's maltose agar.

Colonic contents or feces from 9 dogs and 4 foxes were inoculated into Kauffmann's brilliant green agar after initial enrichment in tetrathionate broth. From only one dog were enteric bacterial pathogens recovered, in this case *Salmonella worthington*. This single isolation is not regarded as having any primary etiologic significance.

No spirochetes were observed in a study of sections of kidney and lymph node stained by the Levaditi method.

As Simms and Muth[4] had been unable to produce infection by use of Mandler (medium) and Seitz filtrates, filtration work was not done at this time. Homogeneous inclusion bodies of characteristic virus type were not found with Pollak's or other methods of staining. Canine distemper and fox encephalitis inclusions were sought particularly.

There was no basis for thinking that the elementary bodies were Toxoplasma or other protozoa.

It appears that the organism belongs to the order Rickettsiales as constituted in Bergey's Manual.[8] Since no arthropod vector is involved and erythrocytes are not parasitized, the organism would fall into the family Chlamydozoaceae. Morphologically and tinctorially the organisms of salmon "poisoning" appear to be identical with the members of this family. They were not cultivated on cell-free media. According to Coon *et al*,[9] and Shaw and Howarth,[10] the disease is effectively treated with sulfonamides, a characteristic of the diseases caused by many members of the group. It is too early to attempt to place the new organism generically. Preliminary work suggests that mice, but not yolk sacs, can be infected.

In many ways the organism resembles *Rickettsia canis* as described by Donatien and Lestoquard[11,12] in Algeria. They believed the infection in dogs was transmitted by the tick, *Rhipicephalus sanguineus*. The incubation period and symptoms reported show a similarity to salmon poisoning except for the absence of diarrhea and vomition.

## Summary

Salmon disease was produced in 6 animals by feeding infected fish, and in 21 by intraperitoneal inoculation.

The principal gross and microscopic lesions in the dog and fox

include hyperplasia of visceral and somatic lymph nodes, sometimes with hemorrhage or necrosis; variable hyperplasia of the spleen, intestinal lymphadenoid tissue, and thymus; and hemorrhage of the gastro-intestinal tract and lungs.

The infection appeared to be established and maintained in mice through three passages.

An organism fulfilling the characteristics of the family Chlamydozoaceae of the order Rickettsiales was present constantly in infected animals, but absent in normal animals.

The disease appears to be the first reported which is caused by rickettsia and transmitted by trematodes.

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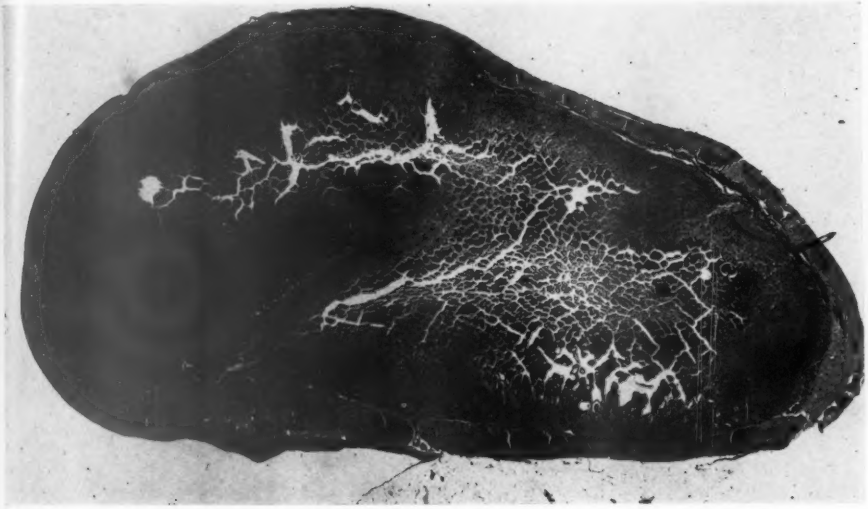
## DESCRIPTION OF PLATES

### PLATE 92

FIG. 1. Fox F-01. Enlarged aggregated lymph follicles of the duodenum. Hematoxylin and eosin stain. × 10. (Armed Forces Institute of Pathology negative no. AC218210-12.)

FIG. 2. Fox F-10. Lungs, showing hemorrhage.



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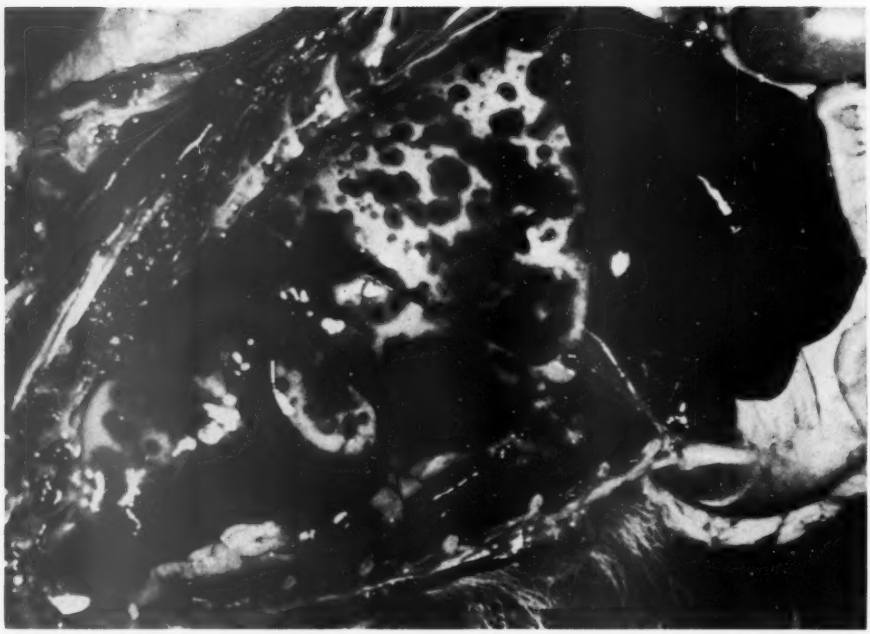

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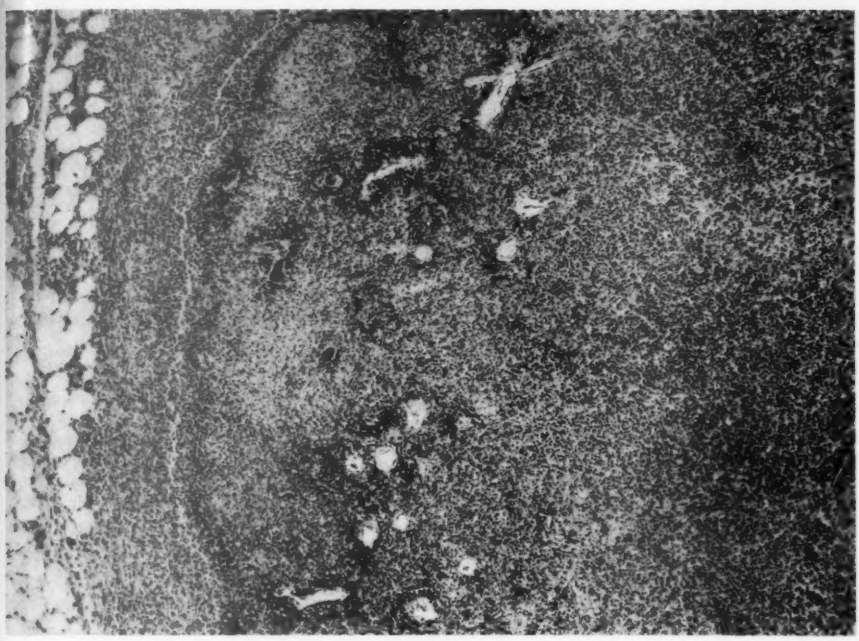
PLATE 93

FIG. 3. Fox F-02. Cortical necrosis in a mesenteric lymph node. Hematoxylin and eosin stain. × 170. (A.F.I.P. neg. Ac218210-11.)

FIG. 4. Fox F-01. Focus of hemorrhage, necrosis, and neutrophils in a splenic follicle. Hematoxylin and eosin stain. × 475. (A.F.I.P. neg. Ac218210-13.)



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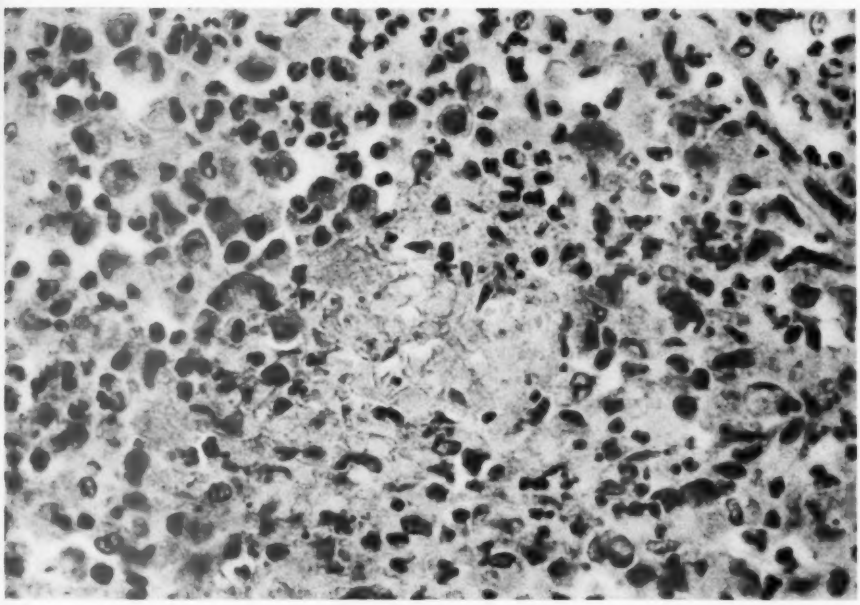

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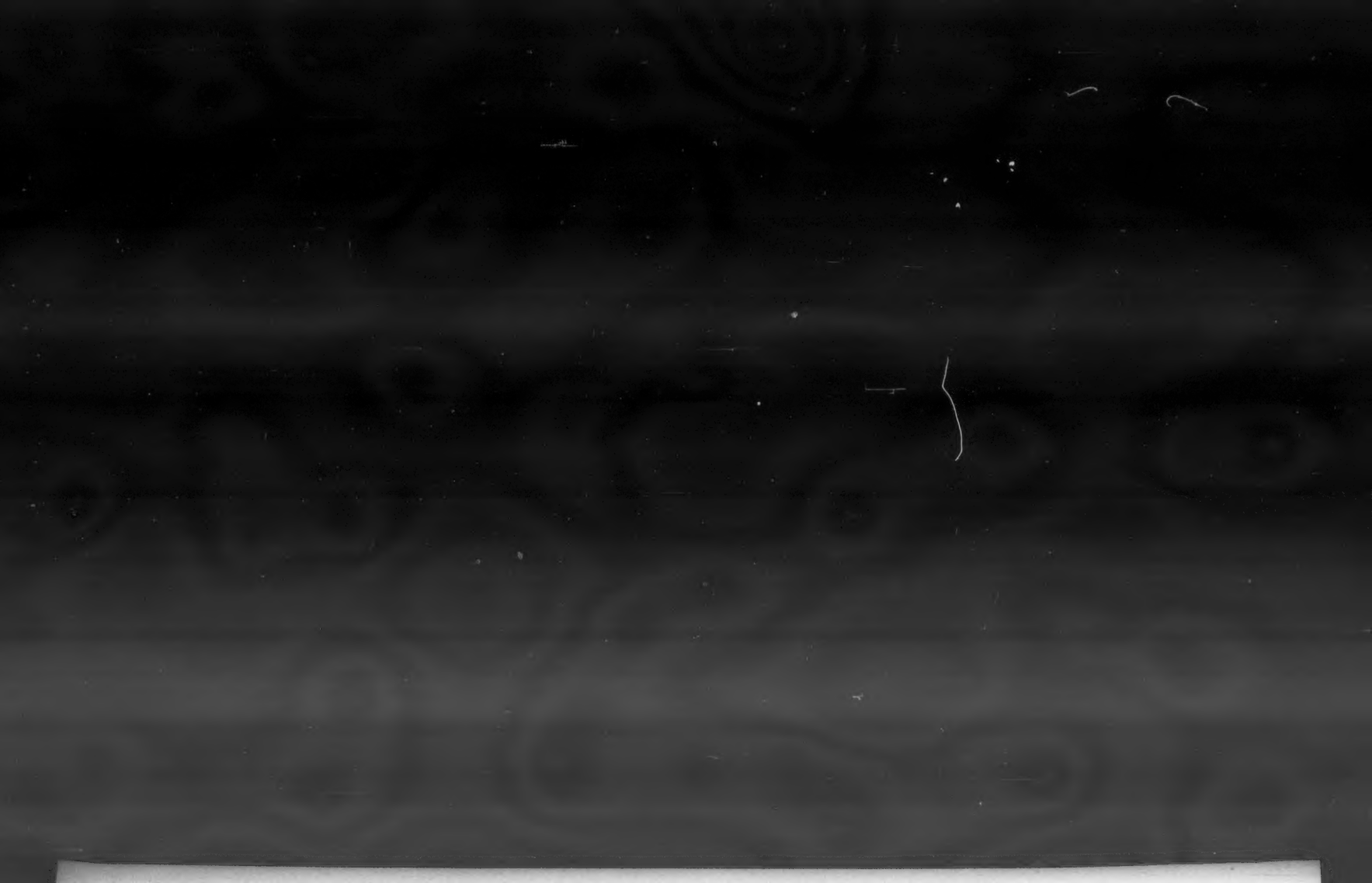
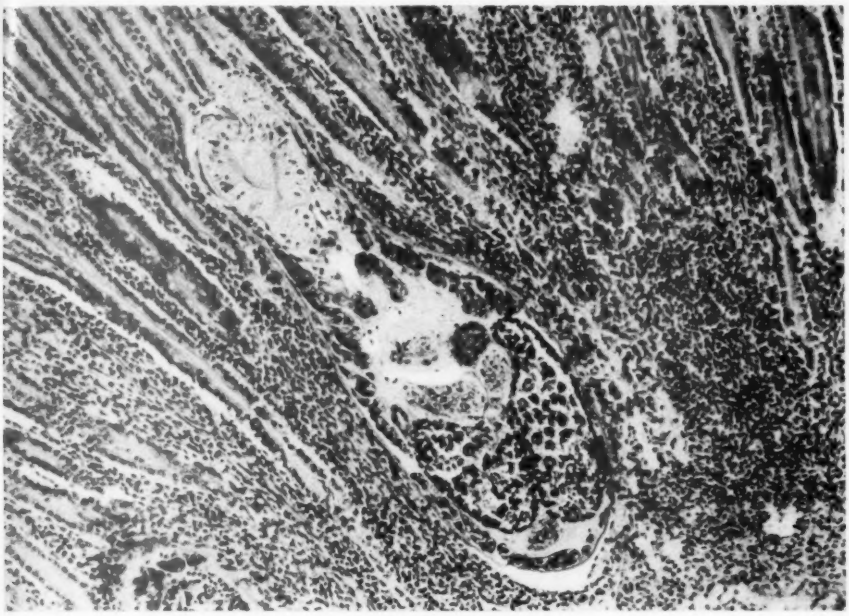
PLATE 94

FIG. 5. Dog D-02. Embedded flukes and enteritis in the duodenum. Hematoxylin and eosin stain. × 114. (A.F.I.P. neg. Ac218210-1.)

FIG. 6. Fox F-02. Elementary bodies in cells of an ileocecal lymph node. Giemsa's stain. × 950.





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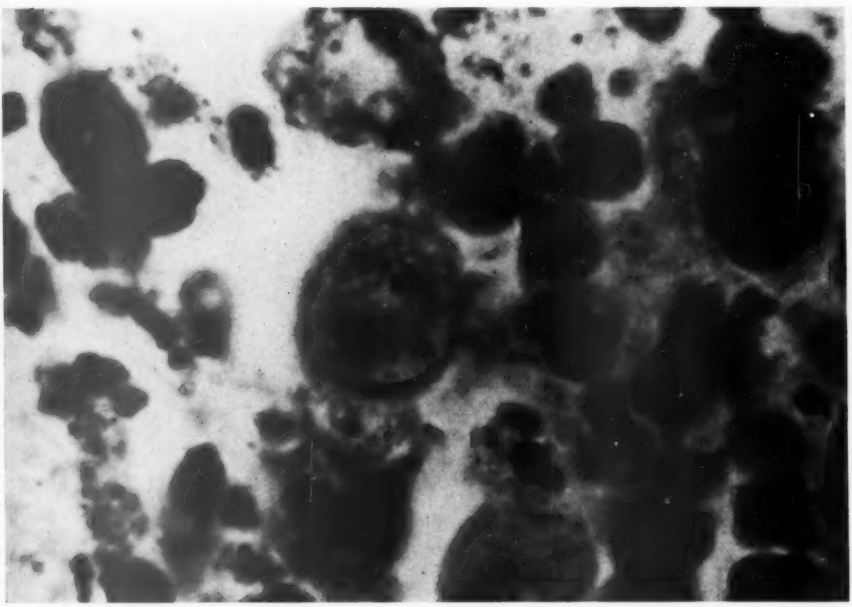

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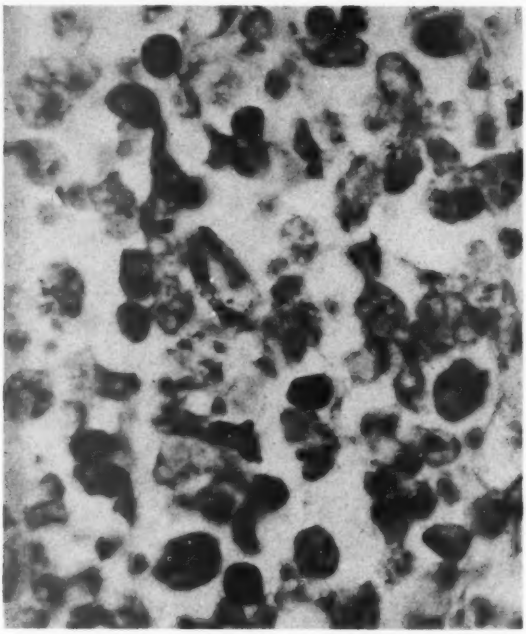
PLATE 95

FIG. 7. Fox F-4. Elementary bodies in cells of a lumbar lymph node. Giemsa's stain. × 440.

FIG. 8. Further enlargement of cells seen in the center of Figure 7. × 1760.



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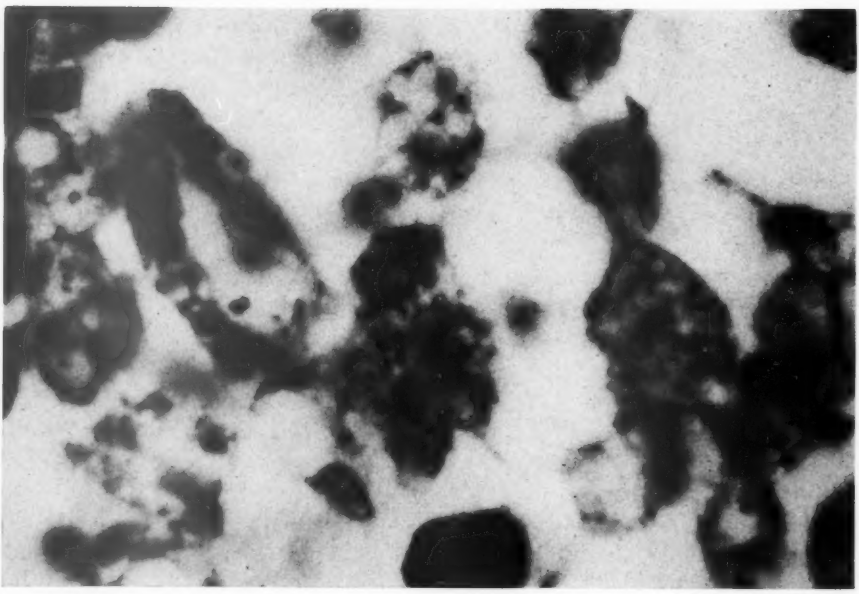

8

# COMBINED HISTOCHEMICAL STAINING OF ACID POLYSACCHARIDES AND 1,2 GLYCOL GROUPINGS IN PARAFFIN SECTIONS OF RAT TISSUES*

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Histochemical staining of polysaccharides has been limited to their affinity for the basophilic dyes,[1] or to their chemical reaction as a class of compounds.[2] There is need for technics which will distinguish individual carbohydrate derivatives or at least separate the classes such as proposed by Meyer.[3] The method of compound staining is as old as the method of staining itself. A combination of histochemical technics on a single slide may well help to add to the knowledge of the location and correlation of the different compounds in the tissues and cells.

In 1946 Hale[4] published a histochemical stain for "acid polysaccharides" (AP) in which he suggested the possibility that the reaction might be due to hyaluronic acid, since pre-treatment with hyaluronidase prevented the staining of the sections to which he applied it.

McManus has published two papers on the use of periodic acid and its application as a histochemical reagent for polysaccharides (PA).[5,6] In 1948 Hotchkiss[7] discussed the chemistry involved, claiming specificity for any substance which contains the "1,2 glycol" grouping or the substituted amino or alkylamino groupings which are not diffused during the course of handling.

These two stains (AP and PA) have been applied by us to sections of many tissues of the albino rat, and, under our conditions, have become the means of staining differentially two classes of compounds which previously have shown identical staining with the basophilic dyes.

The two histochemical stains are applied in addition to our peroxidase stain *in toto*.[8] Each polysaccharide stain, when applied by itself to serial sections, seems to be identical to the other in distribution. Yet, in combination, the two are seen side by side in parallel locations and can be recognized as two distinct chemical reactions. In addition, the peroxidase stain permits the demonstration of granules in appropriate blood cells.

## Method

Fresh tissues were placed in the fixative within 10 minutes. A series of the more common fixatives was tried. There were definite quanti-

* Received for publication, July 18, 1949.

tative as well as qualitative differences in the two histochemical stains in each fixative. We chose the one which gave the maximum preservation of the chemicals we wished to show. A second criterion was the preservation of cell structure as it is demonstrated with hematoxylin and eosin. Ten per cent formalin in 90 per cent alcohol was the fixative which met these requirements. When we applied the peroxidase reaction *en bloc*[8] but omitted the counterstain, better histochemical staining was obtained in that qualitative variations in consecutive rats were at a minimum.

After fixation in alcohol-formol followed by alphanaphthol $H_2O_2$, the tissues were run through paraffin, as described previously.[8] Sections were cut at 4 $\mu$.

### *Combined Staining in Sections of Acid and Periodate Polysaccharides (AP and PA)*

1. Take sections to water in the usual manner

**(AP)**

2. Stain 10 minutes in equal parts of:
   Dialyzed iron (Merck's) and 2 M acetic acid
3. Wash well in distilled water
4. Stain 10 minutes in equal parts of:
   0.02 M potassium ferrocyanide
   0.14 M hydrochloric acid
5. Wash well in distilled water

**(PA)**

6. Flood with 70% alcohol
7. Stain 5 minutes in periodic acid A
8. Rinse in 70% alcohol
9. Reducing rinse, 5 minutes
10. Flood with 70% alcohol
11. Fuchsin-sulfite, 1 hour
12. Sulfite rinse water, 3 rinses
13. Wash well in water

**Optional Counterstain**

14. The routine hematoxylin stain may be used here to stain the nuclei and aid in the identity of the cells. The purple-blue of the hematoxylin can be distinguished from the blue of the acid polysaccharide quite easily.
15. Dehydrate, clear, and mount in dammar

### Preparation of Solutions

*Periodic Acid A*

| | |
|---|---|
| Periodic acid ($H_5IO_6$)* | 400 mg. |
| Distilled $H_2O$ | 10 cc. |
| M/5 sodium acetate (135 mg. hydrated crystalline salt in 35 cc. ethanol) | 5 cc. |

*Reducing Rinse*

| | |
|---|---|
| Potassium iodide | 1 gm. |
| Sodium thiosulfate • 5 $H_2O$ | 1 gm. |

* G. Frederick Smith Chemical Co., Columbus, Ohio.

Distilled $H_2O$ ..... 20 cc.
Then add with stirring:
Ethyl alcohol ..... 30 cc.,
followed by
2N hydrochloric acid ..... 0.5 cc.

*Fuchsin-Sulfite*

Basic fuchsin ..... 2 gm.
Boiling distilled $H_2O$ ..... 400 cc.
Cool to 50° C. and filter. To filtrate add:
2N hydrochloric acid ..... 10 cc.
Potassium metabisulfite ..... 4 gm.
Stopper and let stand in cool dark place overnight
Then add:
Norite ..... 1 gm.
Mix and filter immediately
Add 2N hydrochloric acid up to 10 cc. in small amounts until, after the last addition, the mixture does not become pink when drying spontaneously on a glass slide
Keep in the dark, well stoppered

*Sulfite Rinse Water (Modified Feulgen)*

Distilled $H_2O$ ..... 50 cc.
Concentrated hydrochloric acid ..... 0.5 cc.
Potassium metabisulfite ..... 0.2 gm.

## *Control Staining*

*Acid Polysaccharide (AP).* Because of the involvement of iron in the method, sections were stained by the Turnbull blue technic and the differences compared. The blue-black color of this stain is found in the nuclear membrane of all cells, in a few eosinophilic granules (none in basophils), in a few fine granules in the cytoplasm of the hepatic and intestinal cells, and in the cross-striations of muscle cells.

*Periodic Acid (PA).* A few control sections were run omitting the periodate A as suggested by Hotchkiss.[7] No extraneous staining was ever encountered in our sections, and routine controls were kept at a minimum.

## Location

Table I shows the distribution of the two polysaccharides. Colored illustrations of the combined staining in the kidney, bone marrow, liver, and ileum are shown in Figures 1 to 4. These illustrate the parallelism of the two polysaccharides in the places of higher concentration.

## Discussion

The identities of the staining substances are not known. However, pre-treatment with the enzymes available to us have yielded negative results. Neither stain is decreased after incubation with hyaluronidase.[9] Incubation of the sections with saliva[10] failed to reduce the staining of the reactive substances in all tissues with the exception of

TABLE I

*Distribution of the Two Polysaccharides*

| Tissue or organ | (AP) Acid polysaccharide | (PA) Periodic acid |
|---|---|---|
| Adrenal | Connective tissue, around fat in cortical cells, in cytoplasm of medulla | Connective tissue |
| Blood and bone marrow: | | |
| Eosinophils | Negative | Negative |
| Endothelial cells | In cytoplasm and nucleus(?) | Negative |
| Lymphocytes | Negative | Negative |
| Mast (basophils) | Deep granules in cytoplasm | Negative |
| Megakaryocytes | In cytoplasm and nucleus(?) | In cytoplasm |
| Platelets (smear) | Negative(?) | Negative(?) |
| Red blood cells | Negative | Negative |
| Neutrophile series | Negative | Increasing amounts in cytoplasm from myeloblast through segmented forms |
| Blood vessels: wall | Intercellular substance | Fibers |
| lumen | Fine granules | Negative |
| Bone | In cells | In matrix |
| Cartilage | In cells | In matrix |
| Connective tissue | Intercellular substance<br>Cytoplasm of endothelial cells | In fibers |
| Gastro-intestinal tract: | | |
| Duodenum<br>Cecum<br>Ileum<br>Jejunum | Goblet vacuoles and cytoplasm, brush border and basement membrane, cytoplasm of surface cells and lumen of villus | Lumen, goblet vacuole, brush border(?) and cytoplasm of surface cells of villus |
| Stomach | In cytoplasm of parietal cells, lumen of glands of all layers, and heavy in lumen | In cytoplasm and lumen of middle and surface layers, and heavy in lumen |
| Gonads: | | |
| Ovary | In cytoplasm and nucleus of developing follicular cells surrounding ovum, and in lumen of follicles | In cytoplasm of developing follicle cells surrounding ovum, in lumen of follicles |
| Tube and uterus | In brush border and lumen of glands | In brush border and lumen of glands |
| Testis | In cytoplasm of spermatogonia, in head and tail of sperm | Localized cap around head of spermatids, in neck of sperm |
| Heart: see muscle | | |
| Kidney: | | |
| Cortex | Intercellular substance, cytoplasm and nucleus of glomerulus, fine granules in cytoplasm and lumen of all tubules | In fibers of glomerulus, in lumen and adjacent cytoplasm of all tubules *except* collecting tubules |
| Medulla | In cytoplasm and lumen of all tubules | In fibers, negative in tubules |
| Liver | Fine granules in cytoplasm, in nucleus(?) | Heavy aggregates in cytoplasm |
| Lung | Intercellular substance | Connective tissue fibers |
| Lymph nodes | Intercellular substance | Connective tissue fibers |
| Mast cells: see blood | | |
| Mucous glands | Fine granules in cytoplasm and lumen of glands | Negative |
| Muscle | Intercellular substance<br>Cross-striations | Fibers |
| Skin | Ground substance<br>Hair follicle shaft | Connective tissue fibers |
| Spleen | In cytoplasm of megakaryocytes and endothelial cells | Connective tissue fibers |
| Thymus | Intercellular substance<br>Cytoplasm of endothelial cells | Connective tissue fibers |
| Thyroid | Intercellular substance | Colloid |

aggregates in the hepatic cytoplasm which usually stain after the periodic acid treatment. It would appear from Hotchkiss' work[7] that periodic acid does react with glycogen. The failure of saliva to decrease the staining of the periodic acid reactive substance in all other areas, particularly in the neutrophils of the bone marrow,[11] strongly suggests that these tissues contain a polysaccharide or polysaccharides of similar structure.

TABLE II

*Iron Accumulation in Tissues as Demonstrated by Acid Polysaccharide Stain with Fed Iron Substituted for the Dialyzed Iron of the Usual Technic*

| Tissue or organ | Concentration of iron by AP stain |
|---|---|
| Basophils (mast cells) | Granules through connective tissue of all organs examined |
| Blood vessels | Walls and luminal contents of all vessels |
| Bone marrow | Intercellular substance, endothelial cytoplasm, and basophils |
| Connective tissue | Intercellular substance; many basophilic (mast) granules in endothelial cells |
| Ileum | Goblet cell vacuoles<br>Cytoplasm of glands<br>Basement membrane and brush border<br>Basophils of connective tissue<br>Luminal contents |
| Liver | Intercellular substance, cytoplasm of hepatic cells; luminal contents of bile ducts |
| Spleen | Intercellular substance, endothelial cell cytoplasm |
| Stomach | Cytoplasm and luminal contents of glands, cytoplasm of parietal cells, intercellular substance, basophils of connective tissue (luminal contents of stomach after oral feeding of iron) |

The absorption of dialyzed iron by acid polysaccharide during the staining process suggested to us that this carbohydrate might be concerned in the absorption, transport, or elimination of iron *in vivo*. In a few experiments heavy accumulations of iron were demonstrated in the acid polysaccharide locales when the sections were taken either 3 hours after large oral doses, or 20 minutes after intravenous injection, of iron in different forms. The demonstration of the iron was accomplished by applying the acid polysaccharide technic on paraffin sections with the iron administered *in vivo* substituted for the dialyzed iron of the staining process. However, there was not complete saturation of the AP areas as a small amount of additional iron was taken up when dialyzed iron was applied to corresponding serial sections. The more striking increases in iron in the tissues examined are shown in Table II. The results were identical by both routes of administration of iron.

## SUMMARY

A staining procedure has been developed for the simultaneous demonstration in paraffin sections of polysaccharides of two types.

By this technic the location of polysaccharides of these types in the organs and tissues of the rat has been determined.

By substituting iron administered *in vivo* for the dialyzed iron in the procedure for the demonstration of acid polysaccharides, it appeared that the absorption, transport, and elimination of fed iron is associated with the acid polysaccharides.

We wish to thank Mr. James F. Flaherty for assistance with the histologic sections and Mr. Leslie McWilliam for the colored photomicrographs.

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## DESCRIPTION OF PLATE

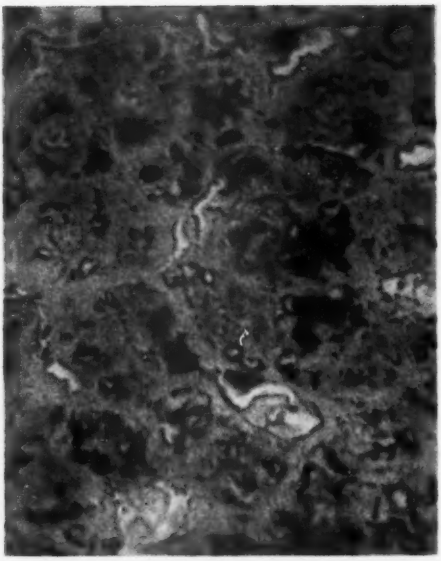
### PLATE 96

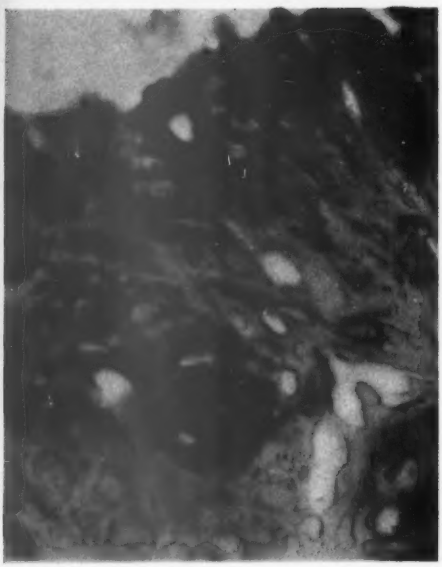
FIG. 1. Combined acid and periodate polysaccharide stains on the rat kidney. A clear separation of the two substances may be noted. × 1200.

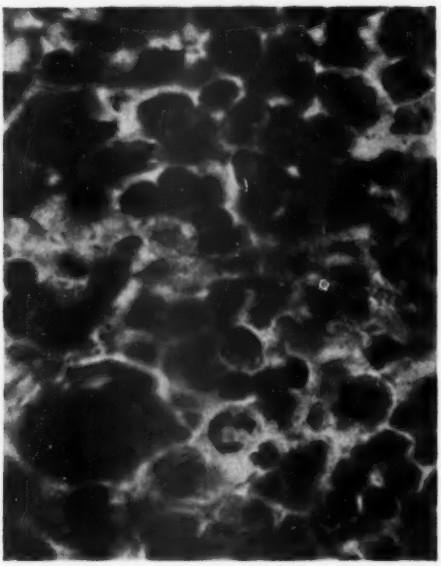
FIG. 2. Rat liver, showing concentration of periodate stain, only part of which is glycogen. (See text.) × 1200.

FIG. 3. Rat ileum, showing distinct separation of the two substances. × 1200.

FIG. 4. Rat femur, counterstained with hematoxylin. Of note are the dark blue basophilic granules, the pink cytoplasm of the neutrophilic cell series, and the distinct staining of cytoplasm of megakaryocytes. × 120.







Ritter and Oleson Staining of Acid Polysaccharides and Glycol Groupings

# HISTOCHEMICAL STUDIES ON TISSUE ENZYMES
## VI. A Difficulty in the Histochemical Localization of Alkaline Phosphatase in Nuclei*

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A technic for staining the sites of action of enzymes which split phosphate esters at approximately pH 9.2 (alkaline phosphatase) has been described by Gomori[1] and Takamatsu.[2] This technic consists essentially of the incubation of paraffin-imbedded sections from acetone or alcohol-fixed tissues in a buffered mixture of a phosphate ester, and calcium ions. In the presence of calcium ions, calcium phosphate is precipitated at those sites where the enzyme acts to liberate phosphate ions. The calcium phosphate thus precipitated is visualized by successive transformation to silver phosphate and metallic silver, the von Kossa technic; or alternatively, to cobalt phosphate and cobalt sulfide.

In a recent paper from this laboratory,[3] three groups of alkaline enzyme activities have been delineated. Group I is the type which most previous authors have described. It splits a great many phosphate esters with approximately equal facility and is inhibited to a great extent by M/4 glycine or arginine, M/100 KCN, by heating sections in water at 80° C. for 10 minutes, or dipping in 5 per cent trichloroacetic acid for 10 minutes prior to incubation. Group II enzymes split only such esters as muscle adenylic acid and adenosinetriphosphate in which the purine riboside-5 phosphoric acid linkage is present. They are inhibited by heat or dipping in trichloroacetic acid, and to a much lesser extent by glycine, arginine, and KCN. Group III enzymes have been recognized only in nuclei. They split esters at varying rates and, unlike groups I and II, are relatively unaffected by heat and trichloroacetic acid under the conditions mentioned above. Like group II, they are affected to a much lesser extent by glycine, arginine, and KCN.

It is obvious that preformed calcium phosphate will be transformed finally to silver, or to cobalt sulfide by the visualizing technics, and the resulting precipitate will be indistinguishable from that resulting from phosphatase activity. A diffuse precipitate distributed uniformly over

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the section and the glass slide between sections has been reported as being due to the activity of enzyme which has diffused from the section into the incubating mixture.[3]

The present study describes observations which suggest the possibility that there may be further limitations to the assumption that every deposit of silver, or of cobalt sulfide, represents a site of phosphatase active during life. The possibility is suggested that under some circumstances staining of some nuclei is an artefact and does not represent the result of phosphatase activity normally present.

## Experimental Procedure

For histochemical studies the tissues were fixed in three changes of cold, absolute acetone, or of 95 per cent ethyl alcohol for 24 hours, then placed successively in absolute alcohol and toluol for 24-hour periods, and embedded in paraffin (melting point, 52° C.). Sections were cut at 10 $\mu$ and mounted using glycerine-egg albumen. The sections were deparaffinized, hydrated, and placed in Coplin jars containing 3 cc. of 0.49 M sodium barbital, 3 cc. of 0.1 M magnesium chloride, 4.5 cc. of 0.085 M calcium nitrate, an amount of phosphate ester to give a final concentration of 0.0033 M phosphate, and distilled water to a total volume of 30 cc. In specified instances, other substances which act as inhibitors or activators were added. The pH of the solution was adjusted to 9.2 using a glass electrode. The slides were incubated at 37° C. for periods specified in each instance. They were then rinsed in distilled water, placed in 0.5 per cent silver nitrate solution under an ultraviolet lamp for 30 minutes, then rinsed in running tap water and in distilled water, and placed in a 2 per cent sodium thiosulfate solution for 2 minutes. They were rinsed in distilled water, stained with eosin, dehydrated, cleared in xylol, and mounted in balsam.

The chemical determination of phosphatase activity was performed by measuring the phosphorus liberated in splitting sodium beta glycerophosphate in a substrate mixture identical with that described above except that no calcium was present.[4] The Fiske-Subbarow method[5] was used, utilizing a spectrophotometer at 660 $\mu$.

## Observations

It has been a rather consistent observation that the nuclei of cells whose cytoplasm stains deeply are themselves stained. Thus, in the muscular layers of the small intestine of the rat, enzymatic activity is noted in irregular patches involving both cytoplasm and nuclei, neither of which are stained in the intervening areas. Moreover, nuclei of cells

at whose surfaces intense enzymatic activity is evident also are likely to be stained even though the cytoplasm of such cells is stained slightly or not at all. Thus, the nuclei of the cells of the proximal convoluted renal tubules stain more darkly and after shorter incubation than those of other portions of the nephron. The most intense enzymatic activity is demonstrated on the brush borders of these same cells. It is further observed that the nuclei of cells adjacent to areas of intense enzymatic activity may be stained deeply even when these cells are of a totally different character than those directly involved in the enzymatic process, and when nuclei of cells of the same character further removed are unstained. For example, Figure 1 represents a portion of human pancreas in which intense enzymatic activity is present in a small duct. The nuclei of parenchymal acinar cells adjacent to this duct are stained, while nuclei of those acinar cells further removed from the intensely stained duct are unstained. Similarly, in Figure 3 of human endometrium, intense staining is noted in the endometrial glands. Those stromal nuclei adjacent to the stained endometrial glands are stained, whereas the nuclei of similar stromal cells further removed are unstained. These observations have been noted in sections of pancreas from 7 guinea-pigs, and in pancreas and endometrium from 2 human autopsies.

A similar phenomenon is noted in other tissues, organs, and species. Its recognition depends on the structure of the organ, and its physiologic phosphatase activity. It will be evident in an organ in which a localized structure is present which shows intense enzymatic activity, and is closely approximated by many cells which do not themselves contain enzyme. Under these conditions, it would be possible to observe nuclear staining of some cells which do not intrinsically contain enzyme and to identify the stained nuclei as those contiguous to the intensely stained local structure. Of all organs studied, those described above most closely approximate these conditions.

However, the phenomenon is noted also in some sections of smooth muscle, as in intestinal wall, where staining of some smooth muscle nuclei adjacent to stained capillaries or nerve plexuses is apparent. The mucosal staining is diffuse, so that it is difficult to recognize such differences in nuclear staining among the fibrocytes of the lamina propria. In brain it is infrequently recognized, since the number of cells surrounding deeply stained capillaries is small, and staining of neuropil is irregular. In kidney, staining of the nuclei of one side of a normally unstained distal convoluted tubule is sometimes evident when that side is contiguous to a deeply stained proximal convoluted tubule. In adrenal, no localized, deeply staining structure is present but it is noted that

nuclear staining is evident beyond the limits of the peripheral zone of intense cytoplasmic staining. In some other organs, the phenomenon is difficult to recognize because of the anatomic and physiologic configuration.

When sections of human pancreas are incubated up to 24 hours in the presence of M/4 glycine (Fig. 2) or arginine, or dipped in trichloroacetic acid, the staining of the ducts (*cf.* Fig. 1) is inhibited. The staining of the adjacent acinar nuclei is similarly inhibited, so that these now stain to the same degree as the acinar nuclei further removed from the duct. All nuclei are lightly stained so that any difference in staining, if present, would not be obscured by intense maximal impregnation. Similarly, when enzymatic activity in human endometrial glands is inhibited by dipping sections in 5 per cent trichloroacetic acid for 5 minutes prior to incubation, all stromal nuclei stain equally (Fig. 4), although longer incubation is required.

When sections of small intestines from guinea-pigs are incubated after having been dipped in trichloroacetic acid as above, the nuclei of fibrocytes in the lamina propria and in the submucosa stain with approximately equal intensity (Figs. 12 and 13).

Deparaffinized slides were placed in a Coplin jar in distilled water at room temperature for periods of 16 hours to 4 days, then stained, together with adjacent serial sections as controls, in the usual buffered substrate solution (sodium beta glycerophosphate in this case) for periods up to 24 hours. It was observed that the former sections showed a decrease in the intensity of staining of the usual sites of cytoplasmic enzymatic activity, and an increase in nuclear staining most clearly evident in areas where there is no staining at all in the control section (Figs. 14-17).

Similar deparaffinized sections were placed in running tap water for periods of 16 hours to 4 days, then stained, together with adjacent serial sections as controls, in the usual buffered substrate solution for periods up to 24 hours. A very marked over-all decrease in staining was observed. There was no increase in nuclear staining.

A series of experiments was performed in which a solution of alkaline phosphatase was added to the incubating mixtures to note the effect on nuclear staining. The alkaline phosphatase solutions were prepared from rat intestine and from guinea-pig kidney by autolysis as suggested by Bodansky.[6] Both solutions gave very similar results in all experiments. When sections were incubated in a buffered (pH 9.2) mixture containing calcium ions and sodium beta glycerophos-

phate with such enzyme solutions added in a final concentration of 1:100, intense nuclear staining was evident in tissues which showed no nuclear staining on adjacent control sections incubated simultaneously in a solution identical except for the absence of such added enzymes. The phenomenon has been observed with concentrations of enzymes as low as 1:1,000. When the enzyme solution was dialyzed, it retained both its phosphate splitting (phosphatase) properties and its nuclear accentuating properties. If anything, the nuclear accentuating properties were more clearly defined since a crystalloid substance was dialyzed out, which in high concentration inhibited and in low concentrations activated all three groups of enzymes.[3] The dialyzed enzymes were heated in a water bath for 10 minutes at 60° C. (Figs. 5 and 6), and retained both their phosphate splitting and nuclear accentuating properties. If heated for 10 minutes at 80° C. (Fig. 7), the dialyzed enzyme solution lost both its phosphate splitting and nuclear accentuating properties. When M/500 iodoacetate or sodium azide was added to the incubating mixture, there was no effect on either property of the dialyzed enzyme solution. When M/4 arginine or M/100 KCN was added to the incubating mixture, both the phosphate splitting and nuclear accentuating properties were inhibited.

Ten slides, each bearing one deparaffinized section of human pancreas measuring approximately 1 cm. by 2 cm. by 10 $\mu$, were placed in a Coplin jar containing approximately 35 cc. of M/500 sodium azide in distilled water at room temperature for 4 days. The sodium azide served to prevent bacterial contamination. A 5 cc. sample of this solution was added to 5 cc. of a sodium beta glycerophosphate substrate mixture, so that the final concentration of substrate, magnesium, and buffer were the same as in the histochemical experiments. The final concentration of sodium azide was M/1000, and the pH was 9.2. No calcium was present. This was incubated at 37° C. and periodic chemical determinations of the phosphorus liberated were performed. The concentration of phosphorus liberated in mg. per 100 cc. was as follows: 0 hours, 0 mg. per cent; 2 hours, 0.163 mg. per cent; 5 hours, 0.212 mg. per cent; 24 hours, 0.330 mg. per cent; 48 hours, 0.554 mg. per cent; 120 hours, 0.885 mg. per cent. Parallel determinations indicate that under these circumstances there is no spontaneous splitting of the substrate. This, together with the shape of the curve, indicates that the phosphorus has been split as a result of enzymatic activity, presumably diffusing from the sections into the solution within the Coplin jar. The absence of bacterial contamination was confirmed by culture.

## Discussion

Our observations on nuclear staining are of significance since they appear to suggest an artefact. In the past, this nuclear staining has been interpreted as evidence of phosphatase activity reflecting the situation present during life. Actually, this staining may represent phosphatase activity which results from changes occurring after death. The observations indicate that nuclei of cells surrounding a focus of intense enzymatic activity are themselves stained more darkly than nuclei of similar cells further removed. Equally significant is the finding that the increased nuclear staining adjacent to a locus of enzymatic activity is due to enzyme of the same group as that of the locus of enzymatic activity itself, and not to group III, the group characteristic of nuclei. In pancreas, the structures showing most staining are the ducts, and in human endometrium, the glands. In each instance, the activity is typical of group I, as indicated by the lack of substrate specificity and the inhibition by glycine and by dipping in trichloroacetic acid. The increased staining of the pancreatic acinar nuclei adjacent to the ducts and of the stromal nuclei adjacent to the endometrial glands, is similarly inhibited by glycine and by trichloroacetic acid, and shows no substrate specificity. When such inhibited sections are incubated for longer periods, nuclear staining is noted which appears to be group III in character since it is unaffected by the inhibitors. Under these circumstances all pancreatic acinar nuclei and all endometrial stromal nuclei stain uniformly. Thus, the *increase* in nuclear staining of the cells adjacent to deeply stained ducts and glands in sections without inhibitors is due to an increase in group I activity characteristic of the ducts and glands to which they are contiguous, and not in group III activity characteristic of nuclei.

A similar interpretation suggests itself with respect to certain observations made in a previous paper[3] on guinea-pig intestine. Figure 8 represents the pattern of enzyme activity when sodium beta glycerophosphate, split by group I enzymes, is used as substrate. The fibrocytic nuclei of the lamina propria adjacent to the stained epithelium are stained, whereas submucosal nuclei at a distance from an area of staining are unstained. Figure 9 shows inhibition of staining in the epithelium and in fibrocytic nuclei in the adjacent lamina propria when M/4 glycine is present in the incubating mixture. Figure 10 illustrates the results when muscle adenylic acid, an ester split by both group I and group II enzymes, is used. In addition to the mucosal staining, staining is present in nuclei and cytoplasm of smooth muscle in both the muscularis mucosae and the muscularis, and in the fibrocytic nuclei in the submucosa which lies between these structures. When glycine is added (Fig. 11), the

staining by the group I enzymes in the mucosa, including staining of fibrocytic nuclei in the lamina propria, is inhibited. However, staining of muscle tissue persists in the presence of glycine, as is characteristic of group II activity. Staining of fibrocytic nuclei in the submucosa encompassed by densely staining smooth muscle also persists. When similar sections are incubated after having been dipped in trichloroacetic acid, a procedure which inhibits both group I and group II activity, the fibrocytic nuclei in the lamina propria and in the submucosa stain with approximately equal intensity with sodium beta glycerophosphate (Fig. 12) and muscle adenylic acid (Fig. 13), indicating that these nuclei show about the same group III activity. Thus, there is an increase in the staining of fibrocytic nuclei above that due to the characteristic group III nuclear activity. Those nuclei in the lamina propria adjacent to areas of group I activity themselves show added group I activity while those nuclei in the submucosa encompassed by tissues showing group II activity themselves appear to show added group II activity.

The evidence presented indicates that there is a difference in enzymatic activity among nuclei under the conditions cited. These differences reflect either the situation present during life or that resulting from changes occurring after death. If the differences noted were ante mortem in origin, they would indicate that certain nuclei which were hitherto considered exactly similar in anatomical appearance, and presumably in function, nevertheless differed in their enzymatic activity. For example, the nuclei of pancreatic acinar cells are usually considered uniform in form and function. The "centro-acinar" cells are really of duct origin and should not be considered. Yet, the nuclei of those acinar cells adjacent to a deeply staining duct stain more deeply than those on the opposite side. Similarly, those stromal nuclei of human endometrium adjacent to the deeply staining glands stain more deeply than those farther removed, although they appear to be identical. The difference in staining of nuclei of fibrocytes in the intestinal lamina propria, from those in the submucosa, is also unexpected in view of their identical appearance and function. It is far more likely that these differences result from changes occurring post mortem, prior to fixation.

This latter concept is favored also by the data which suggest a mechanism by which these changes could occur after death. It has been reported that the particle size of substances showing phosphatase activity is very rapidly decreased after death without loss in enzymatic activity,[7] a change which might facilitate the diffusion of an enzyme fixed in position in high molecular form during life. It has also been suggested[8] that alkaline phosphatase diffuses from tissue sections into the incubat-

ing mixture. This was demonstrated directly by the observation that soaking acetone-fixed tissue sections in water yielded a solution with phosphatase activity. Such diffusion must occur in a radiating fashion from the sites at which the enzyme is normally located, so that the enzyme will be present in higher concentration adjacent to these sites. The nuclei with the added staining are those adjacent to sites of high enzyme concentration, and show activity of the same group.

A second factor is postulated, namely, that nuclei remove and concentrate this diffusing enzyme, so that its activity is manifest within nuclei even though it is not appreciable in the intervening cytoplasm. That this process occurs is suggested by the experiments in which sections were placed for a time in distilled water in a Coplin jar before being stained in the usual fashion. The decreased staining of the sites of cytoplasmic activity is compatible with the postulated diffusion into the incubating mixture, while the increased nuclear staining is compatible with the postulated removal and concentration of enzyme by the nuclei. When slides were washed in running tap water, the latter phenomenon did not occur. Similarly, the experiments in which enzyme solutions added to the incubating mixture resulted in increased nuclear staining also suggest that nuclear concentration may occur. The increased nuclear staining by the enzyme solution is influenced by dialysis, graded heat, and by inhibiting substances exactly as is the enzymatic activity of the solution, suggesting that it is the enzyme itself that is causing the increased nuclear staining.

These findings suggest that many of the previous descriptions of sites of phosphatase activity in nuclei are inaccurate as a representation of the physiologic state. Any conclusions based on such inaccurate descriptions concerning metabolic functions of cells, especially the relationship of nuclear and cytoplasmic functions, may also be inaccurate. For example, many of the observations on which Moog based her discussion of the relationship of phosphatase to nuclear activity in her excellent review[8] may be fallacious.

As this paper was being completed, similar conclusions were published by Jacoby and Martin,[9] based on experiments with superimposition of sections, and on separation of portions of a section rich in enzyme from portions poor in enzyme.

## Summary and Conclusions

Evidence is presented which suggests that the nuclear staining surrounding foci of intense alkaline phosphatase activity, demonstrated

histochemically by the Gomori technic, may be an artefact under some circumstances.

The mechanism by which this artefact is produced may be the diffusion of enzyme after death from the sites in which it is normally present into the surrounding tissues, followed by removal and concentration of this enzyme by nuclei.

Some of the descriptions of sites of phosphatase activity now present in the literature with respect to nuclei may be inaccurate and misleading.

The demonstration of the diffusion of group I and group II enzymes into nuclei does not affect the validity of the group III enzymatic activity in nuclei previously described.[3]

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[ *Illustrations follow* ]

## DESCRIPTION OF PLATES

Where grouped, photographs are of sections from the same block. Unless otherwise stated, sodium beta glycerophosphate at pH 9.2 was used as substrate. The period of incubation and the presence of selective inhibiting or other substances, when used, are specified in each instance. The magnification is 527 × for Figs. 1 to 7, 60 × for Figs. 8 to 13, 527 × for Figs. 14 and 15, and 120 × for Figs. 16 and 17.

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### PLATE 97

FIGS. 1 and 2. Human pancreas, 24-hour incubation. The section illustrated in Figure 2 was incubated in the presence of 0.25 M glycine. In Figure 1, acinar nuclei adjacent to the deeply stained ducts are darker than those farther removed. In Figure 2, where group 1 enzymes are inhibited by glycine, all nuclei stain equally and more lightly, the staining involving nucleoli primarily. This staining is the result of group III activity. An unstained duct is present in the upper left portion of the photograph. The increased nuclear staining in Figure 1 is due to group I enzymes which have diffused from the duct.

FIGS. 3 and 4. Human endometrium. The section illustrated in Figure 3 was incubated for 24 hours. The section illustrated in Figure 4 was dipped in 5 per cent trichloroacetic acid for 5 minutes, washed, and incubated for 84 hours. In Figure 3, endometrial stromal nuclei adjacent to the deeply stained glands are darker that those farther removed. In Figure 4, where group I enzymes are inhibited by the trichloroacetic acid, all stromal nuclei stain equally and more lightly, staining being due to group III enzymes. The increased staining in Figure 3 is due to group I enzymes which have diffused from the glands.

FIGS. 5, 6, and 7. Smooth muscle of human bladder, 6-hour incubation. Figure 5 illustrates a control section stained in the usual manner. Figure 6 illustrates a section incubated in the presence of a 1:100 dilution of a rat intestinal phosphatase solution, which had previously been dialyzed and heated to 60° C. for 10 minutes. The section illustrated in Figure 7 was incubated in the presence of an equal concentration of the same phosphatase solution inactivated by heating to 80° C. for 10 minutes. In Figure 5, it is seen that 6 hours of incubation under these circumstances reveals no evidence of enzyme activity. Figure 6 demonstrates that the addition of an active phosphatase solution to the incubating mixture results in the appearance of evidence of enzyme activity in the nuclei, presumably due to absorption of enzyme by nuclei. In Figure 7 it is seen that when the enzyme activity of this phosphatase solution is destroyed by heat, there is no evidence of enzymatic activity in the nuclei.

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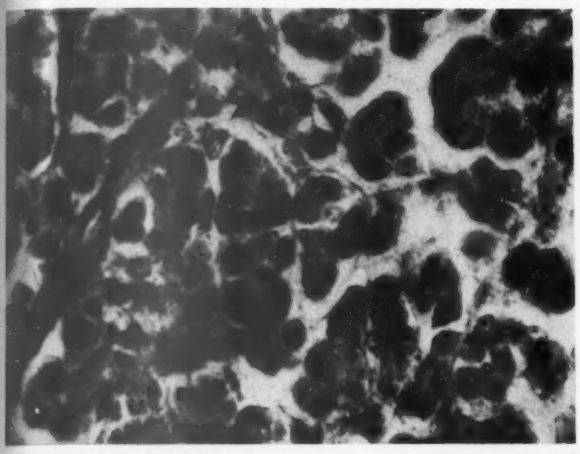

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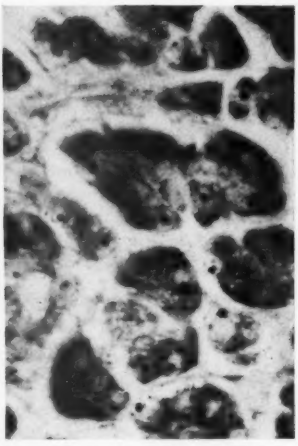

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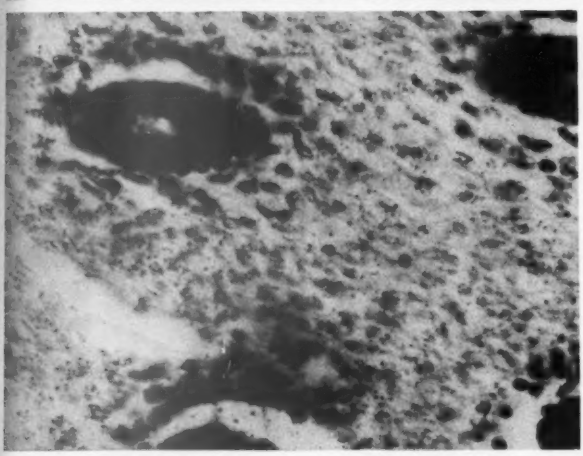

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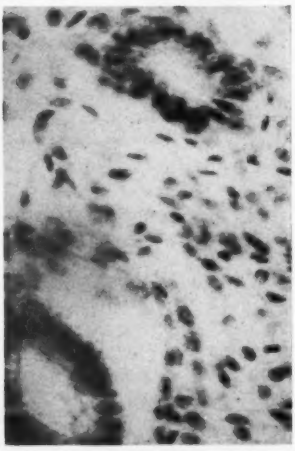

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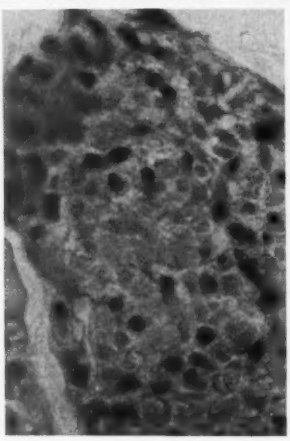

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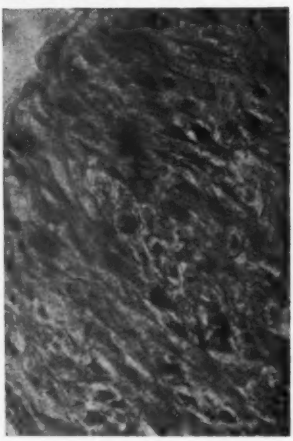

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PLATE 98

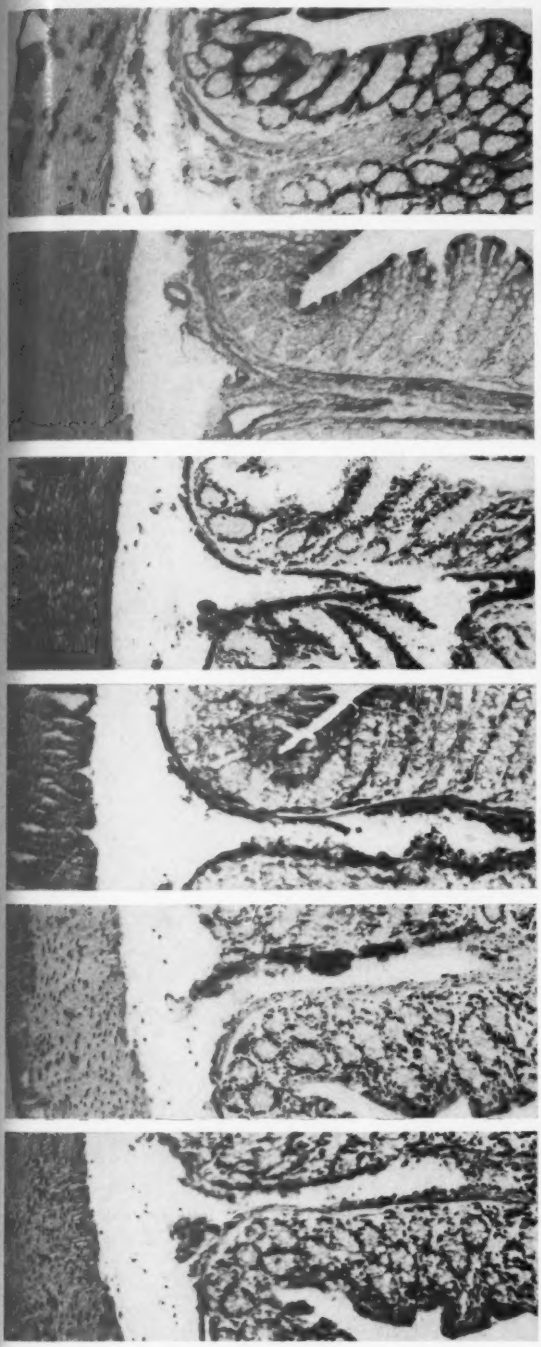
FIGS. 8 to 13. Guinea-pig, small intestine

| | *Substrate* | *Inhibitor* | *Period of incubation* |
|---|---|---|---|
| Fig. 8 | Sodium beta glycerophosphate | None | 24 hours |
| Fig. 9 | Sodium beta glycerophosphate | 0.25 M glycine | 24 hours |
| Fig. 10 | Muscle adenylic acid | None | 24 hours |
| Fig. 11 | Muscle adenylic acid | 0.25 M glycine | 24 hours |
| Fig. 12 | Sodium beta glycerophosphate | 5% trichloroacetic acid, 10 min. | 5 days |
| Fig. 13 | Muscle adenylic acid | 5% trichloroacetic acid, 10 min. | 5 days |

The mucosal staining noted in Figures 8 and 10, including the staining of the epithelium and the fibrocyte nuclei, is due to group I activity. This is confirmed by the inhibition of this staining by glycine, as illustrated in Figures 9 and 11. The staining of the muscle tissue of the muscularis and the muscularis mucosae, and of the fibrocytic nuclei in the submucosa which lies between these structures, as illustrated in Figures 10 and 11, is due to group II enzymes manifested by the use of muscle adenylic acid as substrate. It is noted that some staining persists in the presence of glycine, as in Figure 11. The group III enzymes, present in all nuclei, are equal in the fibrocytic nuclei of mucosa and submucosa, as illustrated in Figures 12 and 13, where both group I and II enzymes are inhibited by trichloroacetic acid. Thus, the nuclei of the mucosal fibrocytes contain group I enzymes derived from the adjacent epithelium while the nuclei of the submucosal fibrocytes contain group II enzymes derived from the encompassing muscle.

FIGS. 14 to 17. Guinea-pig, kidney, 6-hour incubation

Figures 14 and 16 are photographs of the same section, the former at × 527, the latter at × 120. Figures 15 and 17 represent parallel magnifications of a comparable section which had been immersed in distilled water in a Coplin jar for 3 days prior to incubation. The latter sections reveal decreased staining of the proximal convoluted tubules due to diffusion, and an increase, due to absorption, in the staining of the nuclei of the other tubules whose nuclei are unstained in the control section.



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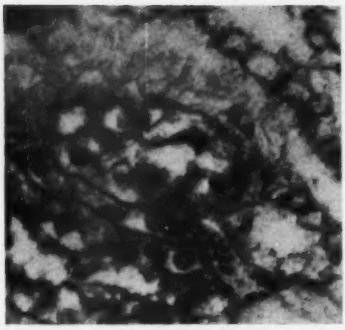

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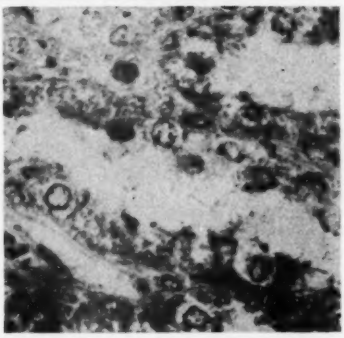

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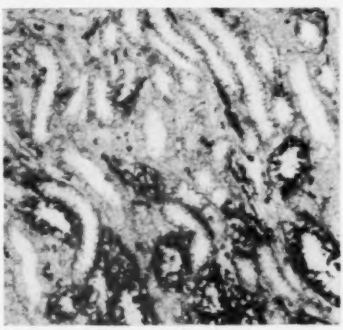

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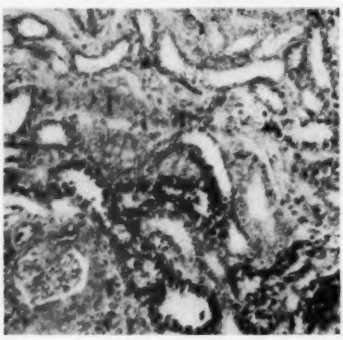

# FORTY-SEVENTH ANNUAL MEETING
# OF THE
# AMERICAN ASSOCIATION OF PATHOLOGISTS
# AND BACTERIOLOGISTS

MADISON

APRIL THIRTEENTH, FOURTEENTH, AND FIFTEENTH, 1950

# THE AMERICAN ASSOCIATION OF PATHOLOGISTS AND BACTERIOLOGISTS

Forty-Seventh Annual Meeting,
University of Wisconsin,
Madison, Wisconsin
April Thirteenth, Fourteenth, and Fifteenth, 1950

PRESIDENT WARREN IN THE CHAIR

## BUSINESS MEETING

April Fourteenth, 1950

Upon nomination of the Council, the Association elected the following officers:

| | |
|---|---|
| *President* | TRACY B. MALLORY |
| *Vice-President* | WILLIAM H. FELDMAN |
| *Secretary* | ALAN R. MORITZ |
| *Treasurer* | SIDNEY FARBER |
| *Incoming Member of Council* | G. LYMAN DUFF |

The President announced that the following officers had been elected by the Council:

| | |
|---|---|
| *Assistant Secretary* | HERBERT Z. LUND |
| *Assistant Treasurer* | WILLIAM A. MEISSNER |

For the Council, the President announced the following actions:

### Election of New Members

Dorothy H. Andersen, New York City
James B. Arey, Philadelphia
Charles T. Ashworth, Fort Worth
Joe M. Blumberg, Augusta, Ga.
H. Davis Chipps, Seattle
Kenneth R. Cross, Des Moines
Joseph A. Cunningham, Birmingham
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Frank J. Dixon, Jr., Brentwood, Mo.
William B. Dublin, Fort Logan, Colo.
Donald E. Fletcher, Little Rock
Lee N. Foster, Indianapolis
Betty B. Geren, Boston
Frans C. Goble, Rensselaer, N.Y.
Peter Gruenwald, Brooklyn
John C. Henthorne, Pittsburgh
Jack H. Hill, Kansas City, Mo.
Abbie I. Knowlton, New York City
Peter P. Ladewig, Montgomery, W.Va.
William L. Lehman, Portland, Ore.
John D. LeMar, Fargo, N. Dak.
Edwin M. Lerner, II, Boston

John J. McGraw, Jr., Bryn Mawr, Pa.
Gardner C. McMillan, Montreal
Albert L. McQuown, New Orleans
Robert C. Mellors, New York City
Ernest E. Muirhead, Dallas
Howard L. Richardson, Portland, Ore.
Simon Russi, Richmond
Jan Schwarz, Cincinnati
Thomas M. Scotti, Richmond
Warner F. Sheldon, Philadelphia
Hilton A. Smith, College Station, Texas
Leon Sokoloff, New York City
Wellington B. Stewart, New York City
Lawrence L. Swan, New Orleans
Alexander Symeonidis, Bethesda
Jerome T. Syverton, Minneapolis
Roger Terry, Rochester, N.Y.
Johannes B. Thiersch, New York City
William W. Tribby, Memphis
Bruno W. Volk, Brooklyn
Stuart A. Wallace, Houston
Levin L. Waters, New Haven
Ephraim Woll, Burlington, Vt.

Reinstatement to membership of Dr. Hou Pao-Chang.

Acceptance, with regret, of the resignations of Drs. George P. Berry, Ellen P. Corson-White, Ruth Gilbert, George M. Lawson, William F. Peterson, M. J. Stewart, Karl M. Vogel, Lewis H. Weed, and R. Lyman Wilbur.

With deep regret, the recording of the deaths of Drs. Samuel H. Gray, S. R. Haythorn, Preston Kyes, Ernst Loeffler, John W. Miller, and Louis C. Posey; and further, of the death of R. Lyman Wilbur, subsequent to his resignation.

The re-election of Dr. Malcolm H. Soule as Assistant Editor of *The American Journal of Pathology* for the ensuing year, and the election of Dr. Ernest W. Goodpasture to the Editorial Board for a period of six years.

The President announced the desire of the Council to arrange for a time and place of future meetings to effect a more economical, less conflicting, and more productive schedule, and invited members at large to offer suggestions for improvements. He stated that representatives of other societies would be consulted and that the Council would give the most serious consideration to the time and place for the next annual meeting; announcement of the final action of the Council will be published in *The American Journal of Pathology* at the earliest possible time.

The President announced that the topic for the symposium in 1951 would be "The Relation of the Adrenal Glands to Systemic Disease," and stated that the name of the referee would be announced in the Journal when the assignment is accepted.

## REPORT OF THE TREASURER

The report of the Treasurer was submitted to the Council and accepted. It was accompanied by a certification from Frank D. Flynn, Tax Consultant, Melrose, Massachusetts. In condensed form, the Treasurer's report follows:

### General Checking Account

*Receipts*

| | | |
|---|---|---|
| Balance on hand, January 1, 1949 | | $ 1,309.12 |
| Membership dues: | | |
| Current year | $ 7,677.55 | |
| Previous year | 20.00 | |
| In advance (1950) | 10.00 | |
| Interest on bonds, from investment account | 500.00 | |
| | | 8,207.55 |
| | | $ 9,516.67 |

*Disbursements*

| | | |
|---|---|---|
| American Journal of Pathology ($8.00 per member) | $ 4,808.00 | |
| C. E. Lennon (Secretary to Dr. Moritz) | 150.00 | |
| P. A. Glass (Secretary to Dr. Farber) | 150.00 | |
| Reporting 1949 meeting | 171.50 | |
| National Academy of Sciences | 250.00 | |
| Officers' expenses at meetings (including printing, badges, travel) | 712.14 | |
| Auditing services | 35.00 | |
| General office expense, secretary | 97.63 | |
| General office expense, treasurer | 234.04 | |
| | | 6,608.31 |
| Balance on hand, December 31, 1949 | | $ 2,908.36 |

*Investment Account*

| | |
|---|---|
| Balance, January 1, 1949 | $34,543.31 |
| Interest on bonds, 1949 | 500.00 |
| Interest from savings banks | 145.47 |
| | $35,188.78 |
| Transfer to checking account (bond interest) | 500.00 |
| Balance, December 31, 1949 | $34,688.78 |

*Inventory*

| | |
|---|---|
| U.S. bonds 2½, series G | $20,000.00 |
| The Provident Institution for Savings | 4,093.99 |
| Franklin Savings Bank | 4,100.80 |
| Cambridge Savings Bank | 4,354.66 |
| National Shawmut Bank | 2,139.33 |
| Total, December 31, 1949 | $34,688.78 |

## SCIENTIFIC PROCEEDINGS

LYMPHOID LESIONS IN POLIOMYELITIS. Frank W. Hartman, Sheldon C. Sommers and (by invitation) Joan C. Wilson, Detroit, Mich.

*Abstract.* The occurrence of striking inflammatory and hyperplastic changes in the lymphoid tissues of intestine, mesenteric nodes and spleen in acute anterior poliomyelitis has received little attention. Study of 50 autopsied cases with acute poliomyelitis, particularly children dying in the preparalytic stage with bulbar involvement, demonstrated a high incidence (82 per cent) of such abnormalities. These consisted of: (1) marked lymphoid hyperplasia, particularly enlargement of germinal centers; (2) very active degeneration and regeneration of reticulum cells in germinal centers; (3) occasional multinucleated giant cells, similar to Warthin-Finkeldey cells in measles; (4) formation of lakes of coagulated protein and nuclear débris in germinal centers, some of the material being phagocytized; and (5) rare intranuclear virus inclusion bodies. Aside from the central nervous system, the lymphoid tissues in poliomyelitis showed the most frequent and severe reactions. The thymus was usually unaffected. The lymphoid lesions are considered of pathogenetic interest and indicative of virus effects. Correlation of these findings with morphologic and virus studies of lymphoid tissues in experimental mouse poliomyelitis, MEF strain, has been attempted.

*Discussion*

(Dr. Shields Warren, Boston, Mass.) I would like to ask Dr. Sommers whether these inclusions have been found in any other cells than of the lymphoid series.

(Dr. Sommers) In experimental and human poliomyelitis, Sabin has reported them in the anterior horn cells. Aside from these cells, in which we also observed inclusions, and the cells of germinal centers, we did not observe inclusions.

FATAL INCLUSION DISEASE IN NEWBORN INFANTS. Frank Vellios (by invitation) and Margaret G. Smith, St. Louis, Mo.

*Abstract.* Inclusion disease (infection with the salivary gland virus) has been observed at autopsy in organs of infants and children in 81 instances, 32 of which have been reported previously by McCordock and Smith. In 3 instances the disease associated with these inclusions, "inclusion disease of infants," appeared to be the cause of death. These patients were premature newborn infants who developed jaundice and petechiae in the skin and mucous membranes. A severe anemia was present in 2. The possibility of blood type incompatibility was investigated in 2, but none was demonstrated. Evidence of syphilis was not present. At autopsy the livers and spleens were enlarged. There was extramedullary hematopoiesis. The characteristic inclusions, both intranuclear and cytoplasmic, were present in the cells of many organs. The salivary glands were not examined. In the kidneys, in each instance, there was focal infiltration of mononuclear cells in the areas where inclusions occurred. Inclusions were present in bile duct epithelium and liver cells. In addition to foci of hematopoiesis there was damage to the livers varying from necrosis of liver cells to definite cirrhosis. Bile thrombi were present in the canaliculi and the smaller bile ducts. In each of the 3 infants inclusions were present in the pancreas, and there was slight intralobular fibrosis. The clinical and pathologic findings were similar in the 3 infants and resembled those reported in stillborn or newborn infants by several other observers. This appears to be the characteristic picture of fatal inclusion disease in the newborn infant, presumably a generalized infection caused by the salivary gland virus. The occurrence of the disease in stillborn infants and in those dying within a few days after birth indicates that the infection may take place during intrauterine life.

In the remaining cases inclusions were present in organs other than the salivary gland in 48 instances. In our experience the disease produced in older infants and children is not clearly defined. In a large group of our series, between 60 and 70 per cent, evidence of this infection has been associated with chronic interstitial pneumonia. In 21 of these pertussis was diagnosed clinically. Diarrhea was the predominant symptom in a small group of patients, and in 3 of these intracellular inclusions were found in large mononuclear cells in the mucosa of the ileum. There is no conclusive evidence in these 48 cases that the inclusion disease was primary, but in some instances the lesions were so widespread as to suggest that the disease was at least a contributing cause of death.

GENERALIZED CYTOMEGALIC INCLUSION DISEASE. J. P. Wyatt (by invitation), St. Louis, Mo.

*Abstract.* Although "inclusion disease of infancy" was described as early as 1904, the intimate linkage between the tissue alterations and the singular viral inclusion-bearing cells has not been stressed. This relationship has been regarded heretofore as rare, fortuitous and inconsequential. On the basis of 5 cases, an incidence of 1.1 per cent in a series of routine post-mortems, factual evidence is presented to support the belief that widely disseminated inclusions, identical to the salivary gland virus inclusion, and linked with morphologic changes highly suggestive of viral effect, represent the lesion of primacy and cause of death in certain baffling illnesses in infants. In living infants, the clinical picture is that of an acute or subacute febrile illness with protean manifestations such as hemolytic disease, hepatocellular jaundice, atypical pneumonia, encephalitis, or acute diarrhea.

These viral inclusion bodies, type B, have been widely distributed, particularly in the lungs, kidneys, liver, and occasionally in the pancreas, adrenal, intestine, and brain. Collateral evidence from comparative pathology and transmission of the salivary gland virus in guinea-pigs substantiate a viral origin of the human disease. The tissues from these infants have shown interstitial pneumonia, nephrosis, viral hepatitis, cirrhosis, and, reported for the first time, extensive necrotizing granulomatous encephalitis and ulcerative colitis with diagnostic viral inclusion bodies. Since the kidney tubules are frequently affected by these characteristic viral inclusion bodies, exfoliative cytologic studies of the urine are suggested as a possible tool for ante-mortem diagnosis. The occurrence of these gigantic inclusion-bearing cells with morphologic changes in a sixth case, a stillborn, is suggestive of intrauterine inception of the disease and that it may be a cause of fetal abnormalities.

TISSUE REACTIONS ASSOCIATED WITH RADIAL INCLUSIONS IN GIANT CELLS. J. A. Cunningham (by invitation), Birmingham, Ala.

*Abstract.* Tissue reactions associated with radial inclusions in giant cells (asteroid or spiculated bodies) have been studied in 23 cases. These include both diffuse disease associated with radial inclusions in giant cells and sporadic focal occurrences. The latter have been seen in both autopsy and surgical material. The histologic and histochemical reactions of the radial inclusions have been studied in an effort to characterize them. They do not appear to be carbohydrate, fat, or nucleoprotein. They can be seen with reduced illumination in unstained paraffin or frozen sections. Studies with microincineration will be reported. Histologic staining shows the material to be acidophilic, staining best with Mallory's phosphotungstic acid hematoxylin, as others have reported.

The radial inclusions are always found in the cytoplasm of multinucleated giant cells, where they are associated with degenerative processes and/or repair. Local fatty retrogressive change, with or without granuloma formation, is often seen in association with radial inclusions; and fat droplets often appear in the cytoplasm of involved giant cells. The association of radial inclusions in giant cells with condi-

tions other than "sarcoid" is emphasized. Ectopic tissue structures and foreign material are often associated with the presence of these inclusions.

*Discussion*

(Dr. Shields Warren, Boston, Mass.) As I recall it, one finds this stellate type of inclusion in some of the inflammatory processes of the salivary gland also, and I think in some specimens I remember a transition from the stellate type to one that has a concentric arrangement. I wonder if Dr. Cunningham in his studies of the stellate bodies has encountered inclusions of other shapes with similar characteristics.

(Dr. Cunningham) Yes, Dr. Warren, we have in several cases found those laminated inclusions. We have not been able to observe any transitions, but in 2 cases we have seen inclusions which looked very much like the so-called Schaumann body, and in several of the cases of sarcoidosis we found laminated inclusions in giant cells and in the same section other giant cells with stellate inclusions.

(Dr. Betty B. Geren, Cambridge, Mass.) I should like to know if Dr. Cunningham has tried to observe these bodies in unfixed preparations or in studies with the phase microscope.

(Dr. Cunningham) We have seen them in unfixed material, but have not examined them with the phase microscope.

THE EFFECTS OF PREGNANCY AND AGE UPON RESISTANCE TO VIRAL INFECTION. John M. Pearce, New York, N.Y.

*Abstract.* In experiments with vaccinia and fibroma virus infections in rabbits the impression has been obtained that not only is the severity of the disease altered by the gravid state but also that in the two diseases the alteration is in opposite directions, vaccinia being milder and fibroma more severe in the pregnant than in the non-pregnant female. In addition non-gravid young animals react much more severely to the fibroma virus than do the old. Controlled experiments were set up to determine the validity of these observations.

Twelve of 24 virginal female albino rabbits, each of which weighed approximately 2500 gm., were rendered pregnant. From 7 to 21 days after conception, 6 of the pregnant animals were inoculated intradermally with serial dilutions ($10^{-0}$ to $10^{-7}$) of vaccine virus and at the same time 6 of the non-gravid females were similarly inoculated. The same procedure was carried out on the remaining 6 pregnant does and their non-pregnant controls, substituting the rabbit fibroma virus for the vaccine virus. Daily thereafter the extent and severity of the lesion at each dilution site and the body temperature were recorded. A similar experiment was performed using the fibroma virus only, and comparing female rabbits approximately 2 months old with does that were over 3 years old. None of these were gravid although many animals in the older group had had litters in the past.

It was found that the dilution at which a vaccinia lesion could be detected and the severity of the lesion at each comparable dilution, as well as the height of the body temperature and duration of fever, were considerably less in the pregnant than in the non-pregnant animal. The reverse was true for the fibroma virus. The lesions produced by it were more severe at each site and occurred at a greater dilution in the gravid animals. A similar more pronounced reaction to the fibroma virus occurred in the young non-gravid rabbits than in the old.

*Discussion*

(Dr. Israel Davidsohn, Chicago, Ill.) These interesting observations can be correlated with the well known fact that serologic tests for syphilis occasionally become much weaker and may become negative during pregnancy. On the other hand, there is the somewhat contradictory fact that certain neoplastic processes are much more

virulent during pregnancy, especially Hodgkin's disease. As to the influence of age, we know that certain natural antibodies are absent in man as well as in animals during the first few months of life. There is a definite pattern of what Hirszfeld called "serological maturation" in which the antibodies absent at birth slowly become increased in titer, reach a peak at puberty, remain stationary until about 50, and then slowly decrease. Whether there is any relation between these observations and Dr. Pearce's observations is a matter of consideration.

(Dr. Shields Warren, Boston, Mass.) I should like to ask whether the increase in female sex hormone may be an important factor or whether resistance may be related to the pregnancy hormones.

(Dr. Pearce) I am sure there is no answer to that. Certainly the pregnancy hormones are growth-stimulating hormones, so that would go along with the increase in the fibroma virus reaction. It is difficult to see how growth-stimulating hormones can prevent the necrosis that occurs in the vaccinia lesion, but is much less marked when the vaccinated animals are pregnant.

A Lipolytic Enzyme in Reactive Histiocytes of Guinea-Pigs with Experimental Encephalomyelitis. F. Stephen Vogel (by invitation), New York, N.Y.

*Abstract.* As a first step towards learning whether a lipolytic enzyme may be a factor in the pathogenesis of experimental encephalomyelitis, a search was made for such enzymes in the inflammatory tissue about sites where brain emulsified with Freund's adjuvants had been injected in guinea-pigs to induce the disease. In 21 guinea-pigs with characteristic lesions of experimental encephalomyelitis, a lipolytic enzyme was regularly demonstrated by the histochemical method of Gomori in the cytoplasm of the large histiocytes that were numerous in the granulomatous tissue about the injection sites. Similar histiocytes containing the enzyme were even more numerous in the regional nodes, and occasional reticulo-endothelial cells in the spleens of the diseased animals also contained it. The same test showed that the lipolytic enzyme was absent from the histiocytes of talcum-powder granulomas in control guinea-pigs, and from the cells of lymph nodes and spleen of normal animals. When an emulsion of brain with mineral oil and falba but without *M. butyricum* was injected intramuscularly into guinea-pigs, the experimental disease did not develop, as previous work had shown, and the same proved true when the injected emulsion contained mineral oil, falba and *M. butyricum*, without brain. Under these circumstances the granulomatous reaction was much less marked, both locally and in the regional nodes, and only an occasional histiocyte gave a positive histochemical reaction for lipolytic enzyme.

Chemical assays confirmed the histologic observations. The inflammatory tissue at the site of injection was removed from 15 guinea-pigs with experimental encephalomyelitis, together with the enlarged regional nodes. The tissues were ground separately with sterile sea sand and a small amount of saline solution; the emulsions were then tested for capacity to hydrolyze olive oil to fatty acid as determined by titration with sodium hydroxide. Each gram of lymph node tissue from animals with encephalomyelitis reacted with olive oil under standardized conditions to yield a mean of 8.8 mg. of oleic acid (range, 5.0 to 11.8 mg.), which exceeded by tenfold the amount of acid formed by the action of lymph node preparations from 12 animals receiving emulsions lacking brain or *M. butyricum* (mean, 0.7; range, 0.0 to 1.3). The granulomatous tissue from the injection sites of diseased animals generally exhibited less lipolytic activity than that of lymph nodes from the same animals. Tissue from the injection sites of 12 animals with encephalomyelitis yielded mean values of 3.0 mg. per gm. of tissue (range, 1.3 to 5.3); this was greater than the lipolytic activity of granulomatous tissue procured from sites where non-pathogenic emulsions had been injected in 12 control animals (mean, 2.2; range, 0.0 to 3.9). The granulomatous tissues of talc-injected guinea-pigs showed no appreciable lipolytic activity when similarly tested. The findings have additional interest in the

light of the demonstration (Vogel, F.S., *Federation Proc.*, 1950, 9, 347) that intracerebral and intravascular injections of purified lipase induce demyelinization in living rabbits.

*Discussion*

(Dr. Shields Warren, Boston, Mass.) Can enzymes having a lipolytic effect other than those from the prepared histiocytes bring about a similar type of lesion?

(Dr. Vogel) The Gomori histochemical stain used to demonstrate the presence of lipolytic enzymes in the histiocytes of guinea-pigs with encephalomyelitis is specific in the sense that a positive reaction is obtained only with enzymes that possess lipolytic activity. This activity may be manifest by many enzymes with varied chemical compositions, but it was not within the scope of these experiments to define further the nature of the enzyme in the reactive histiocytes. Its reaction with olive oil and with brain lipids *in vitro* as well as the results of histochemical staining show it to be similar to pancreatic lipase. In the second portion of the experiment purified pancreatic lipase was injected intracerebrally and intravascularly in rabbits and demyelinization resulted. Other lipolytic enzymes may possibly have a similar destructive effect upon myelin, but they have not been studied as yet.

(Dr. J. A. Cunningham, Birmingham, Ala.) What is the incidence of this demyelinization in clinical cases of acute pancreatitis with high levels of lipase?

(Dr. Vogel) That brings up some experimental work that has shown that when acute pancreatitis is produced in dogs by tying off the pancreatic duct there is increased lipase in the circulating blood, and there is also a deposition of this enzyme in such tissues as the lung which normally do not contain it. Dr. Lewis Stevenson and myself are now studying the material from a young woman who died of acute pancreatitis and whose brain showed demyelinization. These lesions may be the result of circulating enzymes.

THE PATHOLOGY OF FOLIC ACID ANTAGONISTS IN MICE. I. Diamond (by invitation) and S. Farber, Boston, Mass.

*Abstract.* Eight folic acid antagonists given subcutaneously daily for 5 to 30 days produced identical effects at different dosage levels. The significant pathologic findings were marrow hypoplasia, lymphoid depletion, and changes in the intestinal mucosa. The intestinal glandular epithelium showed loss of polarity, anaplasia, and conversion to endothelium-like forms. Glandular distortion and disappearance occurred with resulting mucosal atrophy.

*Discussion*

(Dr. Norbert Enzer, Milwaukee, Wis.) I should like to ask what is the significance of the polylobed leukocytes found in the experimental condition, because I believe the same leukocytes are found in patients treated with the aminopterin group.

(Dr. Diamond) I mentioned that because we have observed it in our experimental material and human cases. It is a finding which has been described also in folic acid deficiency experiments and in pernicious anemia. We believe folic acid deficiency plays a part in the production of these lesions. I say "plays a part" for there is evidence suggesting that there is more than a folic acid deficiency in the production of these lesions.

THE MORPHOLOGIC AND BIOASSAY CHANGES IN ROUS SARCOMA ASSOCIATED WITH DIETARY OR ANTAGONIST-INDUCED FOLIC ACID DEFICIENCY. E. Woll, A. C. Dornbush, and P. A. Little (all by invitation), Pearl River, N.Y.

*Abstract.* Groups of chicks with Rous sarcoma were placed on folic acid deficient diet or were treated with folic acid antagonists. Comparative gross and microscopic studies were carried out at frequent intervals. Samples of the tumor and of the pectoral muscle, the tumor site, were assayed for folic acid content. Constant inhibition of the tumor was associated with folic acid deficiency. This was related

to cytologic changes, similar to those seen in other tissues in folic acid deficiency. Bioassays showed a markedly higher folic acid content in the inhibited tumor than in the tumor site, or the control. The inhibited tumor had a higher folic acid content than the tumor site of the deficient animal.

*Discussion*

(Dr. I. Diamond, Boston) I would like to ask whether Dr. Woll noticed inclusion bodies in the treated Rous sarcoma and if they occur in untreated Rous sarcoma.

(Dr. Woll) The tumor cells often exhibit a phagocytic activity. They may contain broken-down remnants of cells. I prefer to interpret these in terms of cellular débris rather than as inclusion bodies.

THE ASSOCIATION OF LYMPHOCYTES WITH CANCER CELLS UNDERGOING DISTINCTIVE NECROBIOSIS IN RESISTANT AND IMMUNE HOSTS. John G. Kidd and (by invitation) Helen Wallace Toolan, New York, N.Y.

*Abstract.* The cells of three cancers—a lymphosarcoma and two mammary carcinomas—regularly grew progressively when implanted in $C_3H$ mice of the sort in which they originated. Initially they grew quite as well in A mice, forming tumors 1 to 2 cm. in diameter during the first 6 to 8 days after implantation, which proved indistinguishable microscopically from corresponding growths in native hosts. About this time, however, the tumors in the alien hosts abruptly ceased enlarging and within a week as a rule they dwindled and disappeared, the animals then being solidly immune to re-implantation.

In the resistant A mice but not in the susceptible $C_3H$ ones, lymphocytes accumulated regularly and in force about the nodules of proliferating cancer cells. They first became conspicuous after 5 to 7 days and almost immediately penetrated between the proliferating cancer cells, often exhibiting the "tailed," elongated, and bizarre forms manifested by lymphocytes in tissues (Ebert, R. H., Sanders, A. G., and Florey, H. W., *Brit. J. Exper. Path.*, 1940, **21**, 212–218). Soon the lymphocytes are seen in intimate contact with individual cancer cells, one or more of them often indenting the margin of a single neoplastic element or curving like a crescent part way about it. Wherever the lymphocytes have penetrated, but not elsewhere, the cancer cells died one after another, exhibiting as they did so a distinctive sequence of necrobiotic changes. This was characterized initially by a marked increase in cytoplasmic basophilism, which became advanced before the nucleus was altered; later the nucleus became hazy and then more darkly stained, and finally its membrane broke down, so that the contents of the cell ran together and formed a globular mass that stained at first uniformly and intensely with the basophilic dyes, but later unevenly and more lightly; in the stained preparations, the globules often appeared shrunken, and eventually they disappeared without the intervention of phagocytes, leaving empty spaces in the tissue.

These changes differed markedly from the acidophilic coagulation of cytoplasm with pyknotic or karyorrhexic nuclei that characterized necrosis induced in these tumor cells by anoxia or heat. The cancer cells died first at the periphery of the nodules, the process moving inwards as the lymphocytes penetrated, until the last malignant cell was overcome some 10 to 16 days after the implantation. Usually one or more lymphocytes could be found in association with each necrobiotic element; no other inflammatory cell had any such relation to them. Not infrequently, in areas into which the lymphocytes were penetrating, they could be found in close contact with cancer cells as yet unchanged morphologically. When implanted in immune hosts, the cancer cells invariably proliferated during the first 48 hours. Lymphocytes then appeared in force and promptly became associated with the proliferating elements, which then died one after another as in the regressing tumors.

The findings raise two questions: Do the lymphocytes actually kill the cancer cells and bring about the distinctive necrobiosis? Is this an immune reaction affected directly by lymphocytes?

*Discussion*

(Dr. Israel Davidsohn, Chicago, Ill.) The observations on the lymphocytic activity of these $C_3H$ mice are interesting, and I would like to make a few remarks about observations, which I reported last year with Dr. Stern, that may have some bearing on this presentation. The $C_3H$ strain was shown to have low incidence and low titers of natural antisheep agglutinins. This is in contrast to the greater frequency and relatively high titers of natural antisheep agglutinins in the $C_{57}$ black strain. We found that $C_3H$ mice with induced or transplanted tumors showed an increase in the natural antisheep agglutinins. This is of interest in view of the relation between antibodies, globulins and lymphocytes.

(Dr. Alan R. Moritz, Cleveland, Ohio) I would like to ask whether Dr. Kidd has made any *in vitro* observations on the migration of lymphocytes of tumor resistant and tumor susceptible mice in relation to isolated tumor cells.

(Dr. Kidd) We have not studied the *in vitro* migration of the lymphocytes in relation to the tumor cells. When we take the minced normal lymph node tissues of the normal A mouse, and mix this with the tumor cells, there is no effect on the lymphosarcoma cells during 2 hours' incubation; they grow rapidly when implanted into susceptible animals. But if we take the nodes from immune A mice, mince and incubate them with the tumor cells in the test tube, the growth of the tumor cells is inhibited when the mixture is implanted in susceptible animals. Serum from immune animals has no such effect, even when combined with lymph node mince from normal A mice. The observations indicate that humoral antibodies have no discernible part in the immune process.

FACTORS AFFECTING THE NUMBER OF TUMOR METASTASES. EXPERIMENTS WITH A TRANSPLANTABLE MOUSE TUMOR. Morton McCutcheon, Irving Zeidman, and Dale Rex Coman, Philadelphia, Pa.

*Abstract.* There is no relation between the size of human tumors and the number of metastases to which they give rise. Since the reason for this lack of correlation is not known, it is desirable to find the cause. In human cancer there are too many variables for this purpose, such as the variety of tumors, diverse environmental conditions, and different genetic constitutions. Therefore experiments were made with a standardized tumor, mouse sarcoma 241, in an inbred strain, $C_{57}$ black mice. Subcutaneous implants of this tumor always "take" and nearly always produce pulmonary metastases. First, the relation between the number of tumor cells injected into the tail vein and the number of resultant pulmonary metastases was determined. Good correlation was found, although there was evidence of high mortality among the tumor cells injected. This experiment indicates that the number of metastases is proportional to the number of embolic tumor cells given off by the primary tumor. What factors in the primary tumor affect this release of emboli? The factors selected for study in the present experiments were the duration of growth and the size of the primary tumor.

In animals sacrificed from the 9th to the 26th day after implantation of the tumor there was increase in number of pulmonary metastases with time, indicating that duration of growth of the primary does affect the number of metastases. The relation of the initial size of primary tumor to number of metastases was tested by implanting larger (5 mm.) tumor fragments in the flanks of 20 mice, and smaller (2 mm.) fragments in an equal number. When the animals were sacrificed after 17 days, a significantly larger number of metastases (average, 22) was found in mice injected with larger fragments than in those injected with smaller ones (average, 9).

However, the *final* size of the primary tumor was poorly correlated with the number of pulmonary metastases, as is true in human tumors. This result indicates that other, as yet unrecognized, factors are important in regulating the number of emboli released by the primary tumor.

*Discussion*

(Dr. E. J. Eichwald, Salt Lake City, Utah) Do you feel that your conclusions apply to spontaneous tumors?

(Dr. Howard C. Hopps, Oklahoma City, Okla.) What method was used to detect the presence of pulmonary metastases, and what sized metastases might have escaped notice?

(Dr. Shields Warren, Boston, Mass.) There occurs to me one possible point on this matter of size of the tumor. Spontaneous degeneration of implanted subcutaneous tumors occurs very frequently, and I wonder if the foci of necrosis as related to the total volumes of tumor mass were taken into consideration.

(Dr. Zeidman) In reference to the first question—the application of our conclusions to spontaneous tumors—the general impression with spontaneous tumors is that the number of metastases is proportional to the duration of primary tumor growth. Also, it would seem reasonable to assume that there is a direct proportionality between number of metastases and the number of viable embolizing tumor cells reaching the recipient organ. That some tumor emboli may be destroyed in cases of spontaneous tumors has been demonstrated by other investigators, including M. B. Schmidt. The lack of correlation between number of metastases and size of the primary growth is apparent in cases of human tumors, and as yet remains unexplained.

In answer to the second question, the subpleural pulmonary metastases were counted with the aid of a dissecting microscope. Under such circumstances it is possible to miss or misinterpret minute nodules about 0.25 mm. or less in diameter. However, some lung lobes with known gross counts were serially sectioned and the number of metastases was counted microscopically. The microscopic counts were somewhat greater than the gross counts, for a few metastases were in the interior of the lung. Interior metastases were generally fewer than, and proportional to, the subpleural ones. Consequently it is felt that the subpleural metastases count with the aid of the dissecting microscope was a reliable index of the total number of metastases present.

The areas of necrosis as related to the total tumor volume were considered. Single sections through the middle of 60 tumors of known volume were examined. Viable and necrotic zones were outlined, and their respective areas were measured, using a planimeter. In over 95 per cent of the cases, larger tumors had more viable tumor tissue than smaller ones.

THE EFFECT OF HEPBISUL (HEPTYL ALDEHYDE-SODIUM BISULFITE ADDITION COMPOUND) AND THYROXIN ON WALKER RAT CARCINOMA 256.* William H. Kraemer (by invitation), Peter A. Herbut, and John Jacksen (by invitation), Philadelphia, Pa.

*Abstract.* About 10 years ago L. C. Strong first reported on the inhibitory effect of heptyl aldehyde-sodium bisulfite (hepbisul) on spontaneous mammary carcinoma in mice. His results, however, were not duplicated by other American investigators. In our hands hepbisul alone has had an inhibitory action on the Walker rat carcinoma 256, but its salutary effect was only slight. In casting about for a possible catalytic agent, natural thyroxin was used on the premise that it might stimulate the neoplastic cells to more rapid multiplication and thus render them more vulnerable to the action of hepbisul. The results appeared quite promising.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

In the experiment, 108 Sprague-Dawley rats bearing the Walker rat carcinoma 256 were given daily subcutaneous injections of 30 mg. of hepbisul and 0.052 mg. of natural thyroxin per 100 gm. of body weight. One hundred and eight animals, given corresponding amounts of normal saline solution, served as controls. The injections (in the flank opposite the tumor) were started 1 week after transplantation and only tumors measuring more than 15 by 10 by 5 mm. were used. All animals were fed on a routine diet and both treated and control animals were allowed to live at least 3 weeks after transplantation. Of the 108 treated animals, 27 showed complete regression of the tumor and 12 others showed a favorable response (*i.e.*, degeneration and necrosis with replacement fibrosis but with viable tumor cells still remaining). A salutary effect, therefore, occurred in 39 animals or 36 per cent of the total. Of the 108 animals used as controls only one showed complete regression and none showed a favorable response. In another series of 50 animals each, hepbisul alone resulted in 8 per cent complete regression, natural thyroxin 2 per cent, hepbisul and synthetic thyroxin 4 per cent, and saline solution 0 per cent. None of the animals in the second series showed a favorable response. In spite of the large dosage used, local irritation at the site of the injection occurred in less than 50 per cent of the animals and damage to the liver and kidneys was not encountered.

THE EFFECT OF HODGKIN'S DISEASE EXTRACTS ON FERTILE CHICKEN EGGS. Warren L. Bostick, San Francisco, Calif.

*Abstract.* Fertile chicken eggs have been employed in the search for a possible etiologic factor in Hodgkin's disease. Ground extracts from proved Hodgkin's disease lymph nodes have been passed repeatedly through long series of embryonated chicken eggs. Equivalent extracts from non-Hodgkin's disease tissues have been identically passed and used as exactly comparable control observations in all experiments. Early observations of the effect of Hodgkin's disease extracts on chicken embryos (Bostick, W. L. *J. Immunol.*, 1948, 59, 189–193) demonstrated that Hodgkin's disease inoculated eggs showed a distinctly greater mortality than the control series. Although this lethal effect was only slight (11 to 19 per cent greater than in the controls), it is statistically significant ($P = 0.001$ to 0.005). Nonetheless, it was too small a difference to serve as a practical tool for Hodgkin's disease studies. These Hodgkin's disease injected eggs showed no microscopic evidence of any specific lesions.

Extensive procedures were directed towards demonstrating other differences between the control and Hodgkin's disease injected eggs. Exploration of technics of hemagglutination, precipitins, flocculation, etc., revealed no such differences. Complement fixation methods were suggestive but inconclusive in a preliminary survey, so the wide field of virus interference was explored. Since any factor that might be in Hodgkin's disease amniotic fluid gave minimal evidence of its presence, any interference activity would have to be studied by its effect on more easily demonstrable viruses. Those used were viruses that were either quite lethal to chicken eggs or those having hemagglutinative capacities, Lee, PR8, vaccinia, mumps, and Newcastle disease. The study consisted of evaluating the interfering effect that Hodgkin's disease amniotic fluid would have on their growth. The amount of these viruses present was judged by their hemagglutination titers or lethal effects.

Of the viruses studied, the Lee virus seemed most promising. A group of 7-day incubated fertile chicken eggs was first inoculated with 0.1 cc. of Hodgkin's disease amniotic fluid. This amniotic fluid had been harvested from chicken egg series in which Hodgkin's disease lymph node extracts had been originally inoculated 5 to 15 passages before. During these passages, all fluids had at multiple intervals been Seitz-filtered. A group of exactly comparable 7-day incubated fertile chicken eggs was inoculated at the same time with control tissue-extract amniotic fluid. After 3 days' incubation at 37.5°C. all of the injected eggs were inoculated into the amniotic sac with 5 hemagglutinative units of Lee virus that had been harvested from

chicken eggs 2 weeks before and kept in the icebox at 8° C. After inoculation the eggs were incubated for another 18 hours, after which the live ones were removed, placed in the icebox, and their amniotic fluid harvested separately. Each amniotic fluid was titrated separately for the amount of Lee virus present by the hemagglutinative technic (Salk), using human erythrocytes. Fifteen carefully controlled interference tests were performed by the technic described above. The first test showed a clear interference, as did the sixth test. From the eighth test on, every series has demonstrated Lee virus interference, sometimes complete, sometimes partial. No test has ever shown any interfering capacities on the part of the control material. In the 10 positive interference tests, only 27 per cent of the Hodgkin's disease injected eggs revealed any hemagglutination, and their average titer was only 1:390. In the control injected eggs, 62 per cent showed hemagglutination and their average titer was 1:3500. In these experiments the Hodgkin's disease material was originally obtained from 9 patients, and the control non-Hodgkin's disease material was from 4 patients. The above successful interference tests were obtained from amniotic fluid derived from 3 separate groups of patients.

The preliminary experiments described above show that besides the lethal effect of Hodgkin's disease amniotic fluid, it possesses a second property of apparently being able to interfere with the growth of certain viruses in the fertile chicken eggs. Since this second characteristic gives promise of being reproducible, broad avenues for further experimental investigation are obvious, especially in regard to relating directly this virus-like filterable and serially passable interfering factor to the etiology of Hodgkin's disease.

THE COMPARATIVE MORPHOGENESIS OF EXTRAGENITAL AND GONADAL TERATOID TUMORS. Nathan B. Friedman, Los Angeles, Calif., and Washington, D.C.

*Abstract.* Testicular germinomas (seminomas) are tumors of primordial germ cells. Germinomas ripen into germinal carcinomas (embryonal carcinomas), which form somatic and trophoblastic tissue. Chorioepitheliomas and teratomas of the testis, therefore, develop from germinal carcinomas and germinomas. The morphogenesis of ovarian teratoid growths is probably the same. For each of the testicular types enumerated there is a homologous ovarian growth. The incidence of adult teratomas is higher and of teratocarcinomas, chorioepitheliomas, and germinal carcinomas is lower in the ovary than in the testis, a fact which suggests either that development from the same genetic material is influenced by the endocrine environment or that male and female germ cells carry different genetic potentialities for differentiation.

Since germinomas, germinal carcinomas, teratocarcinomas, chorioepitheliomas, and teratomas are all found in both the pineal gland and the thymus, the teratoid growth of these organs should be considered to be of germinal origin. The reasons for the localization of primordial germ cells in these two loci should be investigated. The occurrence of germinomas extragenitally favors the view that the primordial germ cells do not originate from the somatic elements of the urogenital fold. Since this fact also makes it impossible to consider the germinomas as derivatives of spermatic elements, the designation "seminomas" should be abandoned.

Teratomas, chorioepitheliomas, germinal carcinomas, and teratocarcinomas but no germinomas have been observed among the sacrococcygeal teratoid growths. For the present an unequivocal germinal origin cannot be ascribed to the sacrococcygeal teratoid tumors. Many, if not all, of the retroperitoneal teratoid tumors are actually metastases from primary genital tumors and the old observation that such a primary growth may disappear completely should be re-emphasized.

*Discussion*

(Dr. Alexander Symeonidis, Bethesda, Md.) I would like to ask if Dr. Friedman has examined the testes in the mediastinal cases to see if the testes are completely

free of scars because I believe that we must be very cautious when the diagnosis of extragenital chorioepithelioma in man is made.

(Dr. Alfred Plaut, Topeka, Kans.) I have one question concerning the pineal gland tumors. The presence of the large tumor cells and the small so-called lymphocyte-like cells in dysgerminoma, seminoma, or whatever you may call it, gives a picture very similar to that in pinealocytoma with the two types of cells characteristic for the pineal gland. In the pineal gland there is one cytologic feature which distinguishes it from any other organ in the body—the extrusion of chromatin from the nucleus. I would like to ask whether the gonocytoma-like tumors in the pineal gland have been examined for this cytologic detail.

(Dr. Sheldon C. Sommers, Detroit, Mich.) Having had the advantage of examining the normal and abnormal early human material of Hertig and Rock and of seeing pre-villous ova and the early villous stages, I have seen ova with trophoblast which have been examined by serial section and yet nothing has been found which looks like the "germinoma" cell. Therefore it must develop later in embryonic life. That makes it difficult to see how the "germinoma" cell develops into trophoblast. It seems conceivable that teratoid tumors might develop another way, without having intimate knowledge of the matter.

(Dr. Friedman) I am glad that Dr. Symeonidis is here because he is one of those who have pointed out that vestigial teratoid structures and scars in the testis are of significance. I think more testes of men with pineal and thymic teratoid tumors should be carefully studied, although I have failed to find lesions in some. Pathologists are aware that the testes must be studied in "extragenital" chorioepithelioma and I know of one patient with a mediastinal teratoid tumor in whom what at first seemed to be a tiny teratoma turned out to be a hyperplastic hydatid.

These pineal tumors have not been studied from the point of view expressed by Dr. Plaut and that should be done.

Dr. Sommers' fundamental question will have to be answered by the embryologists. All I can do is to point out that there is a monocellular tumor which arises in the gonad and in extragenital sites and seems to have the capacity to develop into other teratoid tumors, including chorioepitheliomas.

TUMORS OF THE PARATHYROID: A REVIEW OF 23 CASES WITH AN EVALUATION OF THE PATHOLOGIC CRITERIA OF CARCINOMA. Lauren V. Ackerman and (by invitation) Boyd Black, St. Louis, Mo.

*Abstract.* The term "parathyroid adenoma" is confined to a tumor involving one gland, usually producing hyperparathyroidism, and which may recur, if incompletely removed, but does not metastasize. Twenty-three cases with long follow-up are presented. It is concluded after summary of other opinions and our cases that oxyphil adenomas do not cause hyperparathyroidism. A unique case of adenomas of multiple endocrine glands with a summary of pertinent hormonal interrelationships is included.

Kidney stones were found in 12 cases. Parenchymal stones suggest hyperparathyroidism. Renal damage is the most important single factor in prognosis and was responsible for 6 of the 9 deaths. Bone changes were present in 17 patients; in 7 a lesion in the maxilla or mandible caused the presenting symptom. Bone changes in primary and secondary hyperparathyroidism may be histologically similar. Secondary hyperplasia of the other parathyroids may result from renal disease caused by hyperparathyroidism. In such cases the entire adenoma should be removed since recurrence of hyperparathyroidism is more to be feared than hypoparathyroidism.

Twenty-six cases reported as parathyroid carcinoma with hyperparathyroidism are evaluated. They are divided into 5 categories. Relative to the 14 cases in the first group with the diagnosis based on cellular characteristics and invasion of the capsule and blood vessels, it is indicated that similar characteristics occur in adenomas. The

case with these changes in the most advanced degree was followed 18 years without evidence of recurrence. In 15 of the 23 adenomas presented, tumor cells were found within blood vessels and in 14, tumor cells were found within the capsule. In the second category consisting of 6 cases with recurrence of symptoms following incomplete removal of the tumor, the appearance of the original tumor was that of an adenoma. A case of recurrence following spilling of tumor at the original operation is presented to emphasize that adenomas are capable of autonomous growth. The third category of tumors are those with evidence of local invasion at the original operation without recurrence of symptoms after removal. The presence of a parathyroid adenoma within the thyroid or thymus does not indicate invasion, but may be an association of the two tissues. The fourth group are those with evidence of local invasion at the original operation and with recurrence of symptoms. In the final group there are 2 cases that presented metastasis and recurrence of symptoms. A third similar case is presented. In this case symptoms have been alleviated twice by the use of roentgen therapy. The term carcinoma of the parathyroid is restricted to tumors with evidence of invasion of muscle or fascia at the original operation and to tumors which metastasize. With these criteria, 6 cases from the literature and a case presented are accepted. A differential diagnosis between adenoma and secondary hyperplasia is presented.

PHEOCHROMOCYTOMAS AND THEIR RELATION TO OTHER CHROMAFFIN TUMORS. H. R. Wahl, Kansas City, Kans.

*Abstract.* This paper is based on a study of 7 pheochromocytomas of the adrenal, 8 paragangliomas (chromaffinomas) of the gastro-intestinal tract, 14 carcinoids of the appendix, and 6 bronchial adenomas of the carcinoid type. All of the adrenal tumors occurred in adults except one in a child 2½ years of age. Three were in adults past 60 and all were benign. Two showed extensive hemorrhages. Pleomorphism was a striking feature in all of the tumors and the cells were well differentiated. The chromaffin and silver reactions were irregular. All tumors except one were in the right adrenal. Of the argentaffin (carcinoids) tumors of the intestine, all were in adults and 4 were in patients of 50 or older. Three were multiple and only one was malignant. Only one was associated with other tumors of neurogenous origin, *viz.*, a schwannoma and a ganglioneuroma.

All tumors studied have two characteristics in common. They are neurogenic in origin and located in the sympathetic nerve tissues and have an affinity for silver salts. Their chromaffin reaction is uncertain. The tumors in the adrenal medulla are composed of differentiated cells whereas the undifferentiated forms apparently predominate in the gastro-intestinal tract and bronchial tree. Hence their relationship is much more difficult to establish. It is possible that the carcinoid is derived from the sympathetic plexus of the intestinal tract. A similar origin is suggested for the so-called carcinoid type of bronchial adenoma, although most evidence does not support this idea.

A study of the development of the adrenal medulla demonstrates the relationship, now well recognized, between the medullary cells and the embryonic neurocytes of the sympathetic nervous system. Theoretically, tumors should develop from an intermediate stage between these two types (neurocytes and adult cells of adrenal medulla), and should present an appearance like the carcinoid of the intestinal tract. No such case has been described, but such should be found. The nearest to it is represented by the partly differentiated tumor cells described in the thoracic tumor of the sympathetic chain by Wahl and Robinson (*Arch. Path.*, 1943, **35**, 571–578). A specific microchemical test for these partly differentiated tumors would aid greatly in clarifying their relationship to each other and to the sympathetic nervous system.

FURTHER STUDIES ON THE HISTOGENESIS OF INTRA-EPITHELIAL CARCINOMA AND EARLY INVASIVE CARCINOMA OF THE CERVIX UTERI. Leland D. Stoddard (by invitation), Cyrus C. Erickson, and H. Lee Howard (by invitation), Durham, N.C.

*Abstract.* In a previous study reported to this Association, proliferation and hyperplasia of reserve cells of the endocervix were traced to a full thickness of metaplastic squamous epithelium, and progressive degrees of cytologic atypicality, to intra-epithelial carcinoma. Material for the present detailed study included 37 additional selected cervices. Thirty-two have been placed entirely in blocks. Thirteen have been methodically serially sectioned, every fifth or every tenth section, as a rule, being taken for study. Ten cases are currently being investigated in this manner.

In all of the cervices there is a field of surface epithelium exhibiting cytologic atypicalities which characterize malignant epithelial neoplasms. This lesion has been referred to by the term intra-epithelial carcinoma *inter alia.* Reserve cell hyperplasia in the endocervix accounts for approximately two-thirds of the lesions, while in the remaining one-third, the field includes both endocervix and squamous epithelium of the portio vaginalis. Usually only a small area of the portio vaginalis at the junction zone is involved, but sometimes the alteration extends far out onto the portio and rarely the field is confined largely to it. It is often possible to distinguish histologically that part of the lesion arising by endocervical metaplasia from that arising by transformation of the squamous epithelium of the portio vaginalis, although squamous differentiation on the endocervical side near the junction may make this difficult. The lesion may be extremely focal or widespread.

Within such a field, invasion into stroma and vessels may occur. Twelve cases are early, invasive carcinomas. One case serially sectioned is of unusual interest because some 50 discrete foci of invasion are present. Some of these invasive foci comprise less than a calculated 50 cells; one had entered a venule and a few, lymphatic channels. The study thus validates the thesis that in some cases intra-epithelial carcinoma represents the pre-invasive stage of an invasive carcinoma, but offers no evidence concerning the frequency of this progression.

NEUROFIBROMATOSIS IN GOLDFISH RESEMBLING VON RECKLINGHAUSEN'S DISEASE IN MAN. Hans G. Schlumberger, Columbus, Ohio.

*Abstract.* Approximately 4 per cent of the goldfish in a large urban pond bear cutaneous neoplasms. Histologic sections from 14 tumors and the *in vitro* growth of the neoplastic cells first suggested that they were leiomyomas. Examination of an additional 38 tumor-bearing goldfish revealed that although a few leiomyomas may occur, the majority of the tumors, whether single or multiple, were neurofibromas and neurilemomas. Of the latter, several showed pronounced nuclear palisading. Occasionally the skin was the seat of a diffuse nodular thickening that resembled somewhat the elephantiasis neuromatosa in cases of human von Recklinghausen's disease. In addition to the cutaneous tumors one fish bore a 4 cm. neurofibroma on the branchial arch; in another goldfish a similar tumor, measuring 6.5 cm. in diameter, almost filled the abdominal cavity. The dermal tumors may be pigmented. The pigment, a melanin, is found in branched melanophores that are intimately associated with the neoplastic cells. Not infrequently neurofibromas arose on the limbus of the eye, where they are comparable to the plexiform neuromas of the upper lid in man. Congenital anomalies of the eye, particularly buphthalmos, may accompany this condition in both fish and man. A number of the affected fish had polycystic kidneys.

Many attempts at transmission of a hypothetical virus by direct tissue transplantation, by contact, or by infestation with parasites have failed to produce the disease.

These negative results plus the presence of associated congenital anomalies, and the rather high incidence of the condition in fish from one pond make it appear probable that in the goldfish as in man neurofibromatosis is a hereditary disease.

*Discussion*

(Dr. Lester S. King, Chicago, Ill.) Were these fish all of the same species, or did they represent various species?

(Dr. Virgil H. Moon, Philadelphia, Pa.) Were attempts made to establish a possible virus origin of these tumors, as has been done with other tumors in lower forms of vertebrates?

(Dr. Schlumberger) As far as species is concerned, they are all of the so-called common goldfish known as *Carassius auratus*. They were placed in the pool 20 years ago, and the caretaker, who is a very observant individual, claims that to his knowledge no other fish have been added. They are of one stock.

Dr. Moon, about the virus, we worked on that notion for 3 years. We had some beautiful ideas. We studied the copepods, which are microscopic crustaceans that bore into these fish, and we thought might produce a tumor by irritation, or perhaps by transmission of a virus; our results were entirely negative.

CHEMODECTOMA IN THE DOG. R. M. Mulligan, Denver, Colo.

*Abstract.* Chemodectoma (chemeia = infusion; dechesthai = to receive; oma = tumor) is a neoplasm consisting of chemoreceptor (chemodector) cells, which are associated with the distribution of parasympathetic nerves and which originate either in the adventitia of blood vessels in structures intimately connected with afferent nerve fibers, or which occur along the branches or in the ganglia of the glossopharyngeal and vagus nerves. In man, sites of chemodectoma may include the tympanic nerve, the adventitia of the superior bulb of the internal jugular vein (Rosenwasser; Lattes and Waltner), and the adventitia of the internal carotid artery (Le Compte) in association with the glossopharyngeal nerve and the nodosal ganglion (Stout) in association with the vagus nerve. In the dog, possible sites of chemodectoma include the adventitia of the aortic arch and of the left coronary, innominate, subclavian, and pulmonic arteries (Bloom) in association with the vagus nerve. Chemodectoma of the aortic arch may be found in man (Lattes and Stout). In man, "paraganglioma" has been used to name "carotid body tumor"; and "nonchromaffin paraganglioma," to designate "jugular body tumor" and "tympanic body tumor." In the dog, "aortic body tumor" has designated the homologous neoplasm of the aortic arch and the base of the heart. "Paraganglioma" is properly reserved for the neoplasm of pressor-elaborating, chromaffin, true paraganglioma cells (J. D. Boyd) of the sympathetic nervous system, found mainly in the adrenal medulla, in interadrenal sympathetic ganglia, and occasionally in thoracic sympathetic ganglia, with "pheochromocytoma" as a synonym. "Aortic body" refers specifically to one of the aortic bodies (organs of Zuckerkandl), collections of chromaffin sympathetic cells around the root of the inferior mesenteric artery. Five male dogs with chemodectoma (three Boston terriers, one fox terrier, and one German shepherd) were 7 to 14 years old. Three dogs showed a single tumor, each on the inner aspect of the aortic arch. The other two had two tumors each. The inner aspect of the aortic arch and the root of the innominate artery were sites in one; the outer aspect of the ascending aorta and the circumflex branch of the left coronary artery, in the other. The tumors were 11 by 5 by 4 mm. (1/5 cc.) to 45 by 30 by 22 mm. (30 cc.), red-gray to tan, and firm. The polyhedral or rounded cells had finely granular, nonchromaffinic, acidophilic cytoplasm and round nuclei, moderately varied in size, with fine, loosely meshed chromatin and solitary, minute nucleoli. The masses of cells were arranged loosely or overlapped and were set in a delicate, vascular connective tissue stroma. The cytoplasm of some cells was marginally clear. Occasional

nuclei were large and bizarre. The neoplasm was closely associated with the adventitia of the affected blood vessel.

INFLAMMATORY PSEUDOTUMORS OF THE PLEURA. W. Jann Brown, Jr. (by invitation) and Lent C. Johnson (by invitation), Washington, D.C.

*Abstract.* In a review of unusual tumors of the chest for correlation with follow-up data, a small group of patients, originally regarded as harboring malignant mesodermal neoplasms, were found to be alive and well as long as 20 years after removal of the tumor. Antecedent respiratory infection, usually pneumonia, was invariably referred to in the histories of these patients. Frequently the first evidence of abnormality was the unexpected presence of a spherical, well delineated nodular shadow in routine roentgenograms of the chest. At operation these lesions were usually situated in interlobar fissures, deflecting the bronchial trees of adjacent lobes, but not invading the lung. The gross specimens consisted of firm, rubbery, rounded masses, which were grayish yellow and sharply circumscribed, and some of them were easily shelled away from surrounding lung tissue.

Microscopically the masses showed numerous hyperchromatic stellate and occasionally polyhedral cells growing between thick bands of dense homogeneous collagen. In many areas it was clear that the cells were fibroblasts producing collagen which in places was thick, lumpy, and irregular as in keloids. Inflammatory cells of all types were present and particularly numerous in the earlier lesions. Because of the bizarre nuclei and occasional mitotic figures in the hyperchromatic cells the tumors had originally been interpreted as malignant mesodermal neoplasms. But in view of the extensive inflammation, marked degree of regularity and uniformity, and lack of evidence of invasion, it seemed probable that the lesions were the result of fibroblastic hyperplasia associated with low-grade inflammation rather than neoplasia. Cases of metastasizing mesothelioma and fibrosarcoma of the pleura and lung, and cases of organizing empyema of the chest cavity were reviewed to determine what features they had in common with the lesions under discussion. Although there were superficial resemblances to the malignant tumors, the histologic pattern was essentially similar to that of an old empyema wall. It is therefore believed that these apparent tumors represent organized effusions, usually interlobar, and the lesions are designated as inflammatory pseudotumors of the pleura.

*Discussion*

(Dr. Paul Klemperer, New York, N.Y.) Do you believe that there are no real fibromas or fibrosarcomas of the pleura? In one of the cases reported by Rabin and myself in which the tumor could be only incompletely removed at operation there was not only recurrence but metastasis.

(Dr. Alfred Plaut, Topeka, Kans.) I have observed one autopsy case which was so similar to the case published by Klemperer and Rabin that the slides could be interchanged. In this case, however, the pleural tumor was a metastasis from a renal carcinoma.

(Dr. Brown) Dr. Klemperer, we have no quarrel with the designation pleural fibroma or fibrosarcoma. There is certainly a group of such cases, as you have pointed out. All we wished to present was the fact that our tumors, which had previously been called fibroma or fibrosarcoma, had not arisen *de novo* but that they were sequelae of a special type of inflammatory effusion.

THE ETIOLOGY OF PULMONARY GRANULOMAS: A BACTERIOLOGIC AND HISTOPATHOLOGIC STUDY OF SURGICALLY RESECTED SPECIMENS. Lyle A. Weed and (by invitation) Lewis B. Woolner, Rochester, Minn.

*Abstract.* With the recent developments of chest surgery an increasing number of pulmonary lesions are being removed. Frequently roentgenologic and bronchoscopic examinations, as well as the cytologic and bacteriologic examination of sputum, fail

to decide the probable nature of the pulmonary lesion so that operative procedures are necessary. Skin tests and serologic reactions are also of uncertain value in determining the specific nature of an infection.

We have examined a group of surgically resected non-neoplastic pulmonary lesions which had the gross and microscopic appearances of granulomas. Grossly they were easily divided into three broad groups: (1) sharply outlined, solid, globular masses with concentric laminations on the cut surface (so-called tuberculoma), (2) solid, irregularly outlined masses with gross and microscopic evidence of extension into the surrounding lung, and (3) lesions that produced necrosis with cavity formation. Specimens of the first group, in general, presented a uniform histologic pattern characteristic of a tuberculoma, with central caseous necrosis and peripheral, more or less palisaded epithelioid cells with giant cells. Bacteriologic examination of such lesions, however, revealed an unsuspected variety of etiologic agents including *Mycobacterium tuberculosis, Brucella suis,* and *Coccidioides immitis.* Additional organisms isolated, of doubtful or no etiologic significance, included *Alcaligenes faecalis* and an unidentified anaerobe. Many of these lesions were negative on culture and animal inoculation.

Lesions of the second and third groups presented histologically a variety of granulomatous patterns including non-caseous (hard) tubercles, caseous foci with epithelioid and giant cells, and tubercles with central collections of polymorphonuclear leukocytes. In certain instances a definitive diagnosis could be made or strongly suspected on histologic grounds alone. In others, however, the histopathologic reaction was non-specific and special stains by Gram and acid-fast technic failed to demonstrate organisms. Cultures of tissues from these lesions revealed such varied organisms as *Coccidioides immitis, Blastomyces dermatitidis, Nocardia asteroides, Mycobacterium tuberculosis* and *Histoplasma capsulatum.* In our experience, acid-fast stains of tissue revealed the organism in approximately half of the cases of proved tuberculosis by guinea-pig inoculation. Spherical organisms in the histologic sections, when found, frequently presented some difficulty in exact identification. This was particularly true in the differentiation of Blastomyces from Cryptococcus and Coccidioides. The endosporulating spherules of the latter are not invariably found in the histologic section, and the smaller endospores in various stages of development may closely simulate Blastomyces and Cryptococcus. One case of coccidioidomycosis occurred in a patient from an endemic area but the skin tests and serologic reactions were repeatedly negative for *Coccidioides immitis. Histoplasma capsulatum* was identified in large numbers in the histologic sections of one proved case; in a second case none could be found with certainty either in sections or smears of the tissue. *Nocardia asteroides* was likewise easily found in certain cases by Gram stain but could not be found in the sections of other cases.

It has been our experience thus far in the bacteriologic examination of pulmonary granulomas that accepted bacteriologic procedures have revealed a wide variety of organisms which frequently were not suspected of being present or which could not be identified with certainty by the conventional diagnostic methods of roentgenologic examination, skin testing, sputum culture or gross inspection and histopathologic study of the removed lesion.

### *Discussion*

(Dr. Virgil H. Moon, Philadelphia, Pa.) This paper reminds us that, at various times in our careers, we may have made diagnoses in rather an offhand manner, which diagnoses, on closer scrutiny, might not prove to be correct.

(Dr. G. J. Dammin, St. Louis, Mo.) I wonder whether from examination of the sections one could have predicted whether the diagnosis might have been made by examination of the sputum.

(Dr. E. Stark, Burlington, Vt.) Were any special precautions necessary in culturing the material, or were these organisms readily found by ordinary methods?

(Dr. Weed) In answer to Dr. Dammin's question I should like to say in some of these cases the diagnosis was made preoperatively by sputum examination, that is, by culturing the sputum. One patient came with the diagnosis of pulmonary histoplasmosis. We examined numerous sputum specimens, and were unable to find the organism by direct examination, but when the material was cultured they were there in large numbers. When the surgically removed specimen was examined by the pathologist he was not able to confirm the preoperative diagnosis by histopathologic studies. The pathologist may many times be put in an embarrassing position if he is not able to confirm the preoperative diagnosis. The case of tuberculosis and the case of blastomycosis were not diagnosed preoperatively, although numerous cultures were made. The 3 cases represented by the tuberculoma-like lesions could not be diagnosed preoperatively. Studies were made, but apparently the organisms were not in the sputum in sufficient quantities to detect them by culture. There are times when one may be able to make a diagnosis preoperatively, but there are many times when one cannot by sputum examination.

The appropriate media used in most bacteriology laboratories were found satisfactory. Brucella may be isolated on ordinary hormone blood agar, but many strains require 5 to 10 per cent $CO_2$. If one is suspicious of a particular type of organism he may have to use the appropriate method to isolate it. With the exception of Actinomyces and Nocardia I find the rest of the fungi will grow on blood agar when 50 units each of streptomycin and penicillin per cc. are added to the medium. These antibiotics will inhibit the growth of bacteria, and the fungi will grow so that one can isolate them in pure culture without difficulty. No one medium will suffice for a routine bacteriologic examination because there are too many organisms that require special technics. I think it is important to have the specimen supplied without contamination whenever possible, to have as large a specimen as possible, and to try as many types of culture medium as available, and even with this, we have had a fair number of cases in which we were not able to isolate any type of microorganism.

STARCH AND STARCH GRANULOMATA. William E. B. Hall, St. Louis, Mo.

*Abstract.* Peculiar irregular round to oval bodies in varying shades of pink have been observed in granulomata of intra-abdominal wounds and perforations as well as in the lung when there has been a history of vomitus aspiration, as well as simple aspiration bronchopneumonia. These bodies are not demonstrable under polarized light. Studies of starch in the processes of digestion with diastase as well as alterations with moisture and heat show starch granules undergo progressive changes ultimately assuming the above forms. In the undigested stage starch presents a typical light pattern under polarized light which is gradually lost in the digestive processes. The observation of these bodies should have a definite medicolegal implication as well as presenting and demonstrating that starch is an actual etiologic agent in the development of pulmonary and perforative granulomata. There is added significance in the observation of persisting starch granules demonstrated under polarized light when the starch has been used as a substitute for talcum powder. The above observations also show that starch is neither digested nor disappears uniformly under the influence of body diastase (amylase), especially if preliminary digestion has occurred in an acid medium.

REACTIVE ABNORMALITIES IN CONNECTIVE TISSUE OF THE LUNG. C. H. Altshuler (by invitation) and D. M. Angevine, Madison, Wis.

*Abstract.* It has been shown that acid mucopolysaccharides (AMP) are generally

formed in the early stages of certain degenerative processes affecting connective tissue and blood vessels. This presentation is concerned with these changes in the lung.

The material studied consists of the following pulmonary lesions from 46 patients:

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| Rheumatic pneumonitis | 10 | Radiation pneumonitis | 1 |
| Disseminated lupus erythematosus | 5 | Interstitial pneumonitis | 4 |
| Periarteritis nodosa | 4 | Primary interstitial pulmonary fibrosis | 1 |
| Systemic amyloid disease | 4 | Primary pulmonary vascular sclerosis | 2 |
| Chronic glomerulonephritis | 4 | Lymphangitic carcinosis | 4 |
| Congenital syphilis | 2 | | |
| Pneumonia of the newborn | 5 | | |

The material was fixed in formaldehyde, embedded in paraffin, sectioned and stained with hematoxylin and eosin, crystal violet, and phosphotungstic acid hematoxylin after chromium chloride mordanting. A modified Sakaguchi reaction for arginine and the Feulgen reaction for desoxyribonucleic acid were used. AMP were demonstrated by their metachromatic reaction with toluidine blue and aldehyde by leukofuchsin after periodic acid oxidation.

Degenerative changes in the lung may be purely local or part of a diffuse systemic disease. In either case, common morphologic features may be shared. These include interstitial edema, fibrosis or sclerosis, alveolar hyaline membrane formation, a variety of degenerative vascular lesions, and certain pleural reactions. These common features are conveniently studied in examples of serous inflammation.

The thickening of the alveolar wall in rheumatic pneumonitis is sometimes associated with a hyaluronidase sensitive metachromatic substance. This is not peculiar to rheumatic fever, for the same change may be observed in radiation pneumonitis, primary atypical pneumonitis, lymphangitic carcinosis, and primary interstitial pulmonary fibrosis. In some instances, alveolar hyaline membrane formation may be seen. This homogeneous, band-like structure lining the alveolar walls has many of the staining properties of fibrinoid. It does not stain metachromatically with toluidine blue but does yield a positive reaction with leukofuchsin after periodic acid oxidation. Sections stained with toluidine blue show interstitial metachromasia and also indicate abundant chromatropic material in the alveoli. A degenerative alteration of small pulmonary vessels also is sometimes associated with the septal change. The vascular lesions in rheumatic or radiation pneumonitis are examples. Occasionally pulmonary vascular sclerosis is prominent in lymphangitic carcinosis and may be of importance in the development of cor pulmonale and right heart failure. For some unknown reason the early serous inflammatory process may be limited to the vessels and results in such conditions as diffuse necrotizing arteritis or primary pulmonary vascular sclerosis. The fibrinoid or sclerotic process in the pleura is similar to that in the alveolar septa.

Since the acid mucopolysaccharides probably participate in the formation of the degenerative structures, their early accumulation is of significance. Although the exact mechanism by which they are transformed is unknown, certain reactions seem probable. Some of these reactions are listed as follows:

(a) Protein reacting as a base + AMP (protein-AMP complex)

(b) Complex protein + AMP (protein-AMP complex + liberated group)
*e.g.*, cephalin-protein + AMP (protein-AMP complex + cephalin)

(c) Hydrophilic colloid + AMP (hydrophilic colloid + AMP)
dispersed flocculent

(d) colloid + AMP (colloid-AMP)
coacervate

(e) Carbohydrate + primary or secondary amine (nitrogen glycoside)

*Discussion*

(Dr. Paul Klemperer, New York, N.Y.) I should like to ask Dr. Altshuler whether he thinks these acid mucopolysaccharides come from the blood plasma. I wonder if in any one of these cases examination for hexosamines was done in the blood serum. Some of the diseases which you have investigated are known to have high hexosamine values in the blood serum. Have you any idea where these mucopolysaccharides come from? It is well known that in granulation tissue mucopolysaccharides are present.

(Dr. Altshuler) We have not determined the serum hexosamine levels in these cases studied. We have felt that the acid mucopolysaccharides in connective tissue are probably formed by the mesenchymal cells. Certainly, materials present in the blood stream may be utilized in the synthesis of the acid mucopolysaccharides, but in a previous study with Dr. Youngner I was not able to demonstrate hyaluronic acid—at least, in its polymerized form—in the blood stream of patients with rheumatic fever, rheumatoid arthritis and a few other conditions. Whether the elevated serum hexosamine levels are of significance in the formation of acid mucopolysaccharides in the connective tissue or not, cannot be stated with certainty at the present time. It is also possible that hydrolyzed polysaccharide gets into the blood stream, or that the elevated serum hexosamine is merely a coincidental finding.

(Dr. Samuel K. Elster, Washington, D.C.) Are you suggesting that the mucopolysaccharides in these abnormal conditions are different from the normal mucopolysaccharides, or are they just increased in amount?

(Dr. Altshuler) We can only suggest they are increased in amount.

(Dr. G. J. Dammin, St. Louis, Mo.) Have you observed hyaluronidase to affect the staining properties of the hyaline membrane?

(Dr. Altshuler) In our hands, hyaluronidase has not affected the staining of the hyaline membrane with toluidine blue. We have not stained the membrane with periodic acid-leukofuchsin after hyaluronidase incubation.

EXFOLIATIVE CYTOLOGY IN PULMONARY TUBERCULOSIS. H. Davis Chipps (by invitation), Louis H. Krauel (by invitation) and S. W. Lippincott, Seattle, Wash.

*Abstract.* Several investigators have utilized the Papanicolaou smear method, or modifications of that technic, in studying exfoliated cells from various orifices and cavities of the body. In general there has been endorsement of this method as a valuable aid in the diagnosis of bronchogenic carcinoma with perhaps too little recognition of certain inflammatory lesions that offer diagnostic problems. The objective of this study was to investigate the latter.

This study has been performed on sputums and/or bronchogenic washings from 240 cases of proved, active tuberculosis presenting various stages of development, complications of the disease, and response during different types of therapy. Many of these patients were followed with multiple specimens for as long as 3 years, and none less than several months. None of these individuals developed or had a bronchogenic carcinoma, and yet, often in serial studies, exfoliated cells were found showing the atypical, cytologic features that have been ascribed to neoplastic cells. These features were associated principally with alterations in the nucleus, consisting of hyperchromatism, anisokaryosis and clumping of chromatin.

*Discussion*

(Dr. Walter Putschar, Charleston, W. Va.) I should like to ask if, in some of these cases, the diagnosis of tuberculosis had not been known, would malignancy have been suspected.

(Dr. Paul Klemperer, New York, N.Y.) Were similar atypical cells found in other inflammatory conditions of the lung than tuberculosis?

(Dr. Norbert Enzer, Milwaukee, Wis.) Were comparative studies made by paraffin block section and by the smear technic?

(Dr. Chipps) I should make it clear that this discussion was taken out of the context of a much larger examination of bronchial material and represents a control group of known tuberculous cases. Further control groups included non-tuberculo- inflammatory lesions, such as bronchiectasis, a normal control group, and finally a group in which a diagnosis of carcinoma had been made. I am not prepared at this time to give the findings on these other groups. In seeing these selected cells, and they were selected, I would certainly suspect some of them of being from carcinoma, but I would like to indicate that I would not make a diagnosis of carcinoma unless I knew something about the case and had followed it, and, if possible, had confirmed a suspicious diagnosis by conventional biopsy methods.

In regard to finding atypical cells in other material, we did find atypical cells and we found also the Herbut-Clerf clusters. These probably are due to ulceration of the mucous membrane and probably are not pathognomonic of tuberculosis, although they are more common in tuberculosis than in any other lesion.

In regard to the question about block and smear comparison, I have already said that in these cases we did concurrent examinations of blocks and of smears. I believe I said that sometimes the block proved superior and I know some people prefer it. In my own practice, if I were limited to one method, it would be the smear, although actually I prefer to do both.

THE REACTION OF RETICULO-ENDOTHELIAL CELLS OF RABBITS TO TOTAL BODY X-RADIATION. J. Barrow (by invitation), John L. Tullis, and F. W. Chambers (by invitation), Bethesda, Md.

*Abstract.* There have been conflicting reports in the literature concerning the relative radiosensitivity of reticulo-endothelial cells. Histologic examination of the tissues of animals subjected to x-rays from a high energy source and to penetrating radiations from an atomic bomb source reveals little or no morphologic alteration in the reticulo-endothelial elements. Further, it is evident that phagocytosis, as demonstrated by erythrophagocytosis and by the prompt removal of cellular débris, is active in animals so treated. However, Chrom and Becker, in particular, have indicated that reticulo-endothelial cells in the irradiated animal may be damaged functionally.

In an effort to demonstrate alteration in the phagocytic properties of the reticulo-endothelial system of animals subjected to total body x-radiation, rabbits were injected intravenously with radioactive colloidal gold mixed with appropriate amounts of stable non-radioactive colloidal gold so as to provide a mixture which would give 7 cc. in total volume and 25 microcuries of radioactivity per kg. of body weight. The particles of colloidal gold were remarkably uniform in size (average 275 Å) and were chemically and physiologically inert. Blood samples obtained by cardiac puncture in control gold-injected rabbits, in x-radiated gold-injected rabbits, and in the same rabbits gold-injected before and after x-radiation were surveyed for radioactivity with a Geiger-Müller counter. No significant differences in the rate of disappearance from the blood stream of the colloidal material as a result of total body x-radiation in single doses of 500 to 800 r. have been observed.

That the rate of disappearance of the radioactive material from the blood stream is tantamount to the rate of uptake of the colloidal material by the cells of the reticulo-endothelial system, was determined by surveying with a Geiger-Müller counter portions of the ground-up organs of the experimental animals. Liver, bone marrow, spleen, and lungs together contained from 75 to 90 per cent of the total radioactive material injected. The liver alone contained 58 per cent or more of the total dose injected. Review of the histologic sections demonstrated the concentration of colloidal material within the reticulo-endothelial cells. From these data it is

concluded that exposure of the total body of rabbits to single doses of 500 to 800 r. of x-radiation fails to damage the phagocytic function of the reticulo-endothelial cells or alter the rate of phagocytosis of colloidal gold particles in the circulating blood.

HISTOCHEMICAL AND MICROCHEMICAL STUDY OF MOUSE LIVER FOLLOWING A SINGLE FEEDING OF CARBON TETRACHLORIDE. Robert E. Stowell, and (by invitation) C. S. Lee and K. K. Tsuboi, Kansas City, Kans.

*Abstract.* Histochemical studies of alkaline phosphatase, esterase, glycogen, lipids, desoxypentose and pentose nucleic acids were compared with microchemical studies and with quantitative data on the mean volumes of necrotic cells, vascular spaces, hepatic cells and their nuclei, nucleoli, and cytoplasm at intervals up to 18 days after a single feeding of carbon tetrachloride. During the first day, frequently before necrosis is demonstrable by hematoxylin and eosin staining, there is a decreased staining reaction for alkaline phosphatase and pentose nucleic acid in the central part of the liver lobule. As this early evidence of cellular damage progresses to recognizable necrosis after the second day, the esterase, lipids, and desoxypentose nucleic acids also decrease in the dead cells. The remaining cells in the periphery of the lobules contain more glycogen and less lipids than normal fasting liver. During the third to sixth day after feeding carbon tetrachloride there is a marginal zone of cells between the decreasing central necrotic areas and increasing peripheral viable cells. This marginal zone contains cells with lipid-filled cytoplasm and scant glycogen. At the fourth to sixth day regenerating cells contain an increased number of nucleoli with more intense pyronin staining, a finding consistent with the presumed rôle of the nucleoli in nucleic acid and protein synthesis within these cells.

As compared with controls, microchemical measurements of homogenized liver on the second day, when central necrosis comprises 37 per cent of the tissue, showed decreases of total esterase of 52 per cent, acid phosphatase of 12 per cent, succinic dehydrogenase of 27 per cent, pentose nucleic acids of 13 per cent, and desoxypentose nucleic acids of 19 per cent. During the third to sixth days there was an average increase in the same constituents of 86, 55, 20, 58, and 53 per cent, respectively. Alkaline phosphatase, which has already increased 15 per cent by the second day, is augmented 103 per cent by 4 to 6 days. The total lipids decrease 44 per cent by the fourth to sixth days. Most constituents measured approach their normal value by 18 days after carbon tetrachloride feeding.

This investigation illustrates a coordinated approach to the interpretation of changes in structure and chemical composition of liver cells during necrosis and subsequent repair.

*Discussion*

(Dr. Samuel K. Elster, Washington, D.C.) Was there any difference in the microchemical pattern of the animals that survived and the animals that died as a result of carbon tetrachloride poisoning?

(Dr. Stowell) We did not undertake an extensive series of observations on the effect of different doses of carbon tetrachloride. The amount which was used was sufficient to produce liver damage, but not death. We did make a few observations in which twice the usual dosage or 0.08 cc. of carbon tetrachloride in 0.1 cc. of olive oil was given. In these animals a decrease in pentose nucleic acid the first day after feeding was much more evident than in the other experiments. The early decrease in the enzymes studied was accompanied by extensive necrosis of the central part of the liver lobule.

THE STRUCTURE OF MITOCHONDRIA AND ITS RELATION TO THE CYCLOPHORASE SYSTEM OF INTEGRATED ENZYMES. John W. Harman, Madison, Wis.

*Abstract.* Mitochondria were obtained by homogenization of rabbit kidney, liver, and heart in either 30 per cent sucrose or 0.9 per cent KCl at 0° C. Mitochondria in

30 per cent sucrose were rod-shaped, those in saline solution were enspherulated. Manometric studies on mitochondrial suspensions, either purified by differential centrifugation or mixed with chromosomes, invariably demonstrated cyclophorase activity. The cyclophorase system of integrated oxidative enzymes was intimately associated with mitochondria. This was apparent because: (1) The destruction of the mitochondria by transforming agents accomplishes disintegration of cyclophorase; (2) both the mitochondrial counts and form of the mitochondria influence the rate of oxidation; and (3) the integrity and distribution of the mitochondria determine the occurrence and distribution of the cyclophorase in the various fractions obtained by differential centrifugation.

The spherical mitochondria in 0.9 per cent KCl had a composite structure, clearly depicted by phase microscopy. They consisted of a dense peripheral crescent and a transparent residue; the crescent represented the site of the original rod. Exposure to de-ionized water and 30 per cent urea caused swelling and disappearance of the internal differentiation, with simultaneous loss of cyclophorase activity. Suspension in hypertonic solutions caused shrinkage, with accentuation of the crescent. The reversible swelling and shrinkage of mitochondria by variation of tonicity suggested an osmotic system. Experiments were designed to detect a semipermeable membrane. Aliquots of cyclophorase suspensions (containing both mitochondria and chromosomes) in 0.9 per cent KCl were resuspended in de-ionized water, 30 per cent urea, 0.85 per cent NaCl, and in fresh 0.9 per cent KCl at 0° C. After 30 minutes the particles were separated by centrifugation and the soluble protein in the supernates determined. These drastic lytic agents released neither protein nor nucleoprotein from the mitochondria or the chromosomes. Neither structure has a membrane of zero tension such as exists in the red blood cell.

In other experiments the behavior of radioactive ions was studied. Cyclophorase suspensions were mixed with solutions of radioactive sodium and potassium. The suspensions were then centrifuged and the radioactivity of both residue and supernate was determined. The activity was the same in each. The mitochondria and chromosomes offered no barrier to penetration and no tendency to accumulation of the ions. All preparations were enzymatically active and oxidized alpha ketoglutarate to $CO_2$ and water. The mitochondria are regarded as macromolecules, with a folded lattice structure and properties of a fibrous gel. This would explain the osmotic peculiarities most precisely. This gel contains an integrated complex of enzymes, the cyclophorase system of Green, which implements the Krebs citric acid cycle and represents a common oxidative pathway in the cell. Mitochondria are essentially intracellular "furnaces" for the complete oxidation of fatty acids, amino acids and components of the Krebs cycle.

*Discussion*

(Dr. Betty B. Geren, Cambridge, Mass.) I should like to know how Dr. Harman interprets the observations made by others, most notably Dalton and his group, on membranes seen in the electron micrographs of mitochondria in solutions of varying tonicity.

(Dr. Harman) The observations made by use of electron microscopy have interesting results, it is quite true. However, an observer in our institution made attempts to take photographs, which we think can be interpreted in another manner than that of Dalton and which support the type of structure we have observed here. We observed the star-like structures which were interpreted as a clumping of the internal structure. Another thing is that we found it very difficult to understand how Dalton explained the material he saw coming out of the mitochondria as unequivocally the contents of that body. Our results with washings are against his view. These have been repeated, again by another observer in our laboratory, and we have failed to detect a significant amount of soluble protein. It is true, on the other hand, that Claude obtained soluble protein from suspensions of mitochondria. I would like to

point out in this respect, however, that he let his preparations sit around for 24 hours, and that autolysis might explain his results adequately. We believe that a half-hour exposure to de-ionized water would have accomplished elution of soluble protein if the units were bounded by semipermeable membranes.

(Dr. Geren) What order of magnitude are these radiating structures you see?

(Dr. Harman) It really is like a crenation such as you see in the red cell. If you took drops of protein or gel and dropped them on a slide and photographed them, the effect is similar. So it is unnecessary to implicate a semipermeable membrane when the phenomenon is explicable by alteration of gel structure.

(Dr. Geren) You did not see any evidence of three-dimensional lattice structures?

(Dr. Harman) Not in the pictures we have taken.

STUDIES OF THE PATHOGENESIS OF HUMAN ARTERIOSCLEROSIS AND EXPERIMENTAL ARTERIOSCLEROSIS IN THE PYRIDOXINE-DEFICIENT MONKEY. James F. Rinehart and (by invitation) L. D. Greenberg, San Francisco, Calif.

*Abstract.* In 1948 we first reported the occurrence of arteriosclerotic lesions in rhesus monkeys subjected to pyridoxine deficiency. Some details of the findings were published in 1949. Since this time the work has been extended, and we have undertaken a careful study of the evolution of the lesions. A prominent feature of the process has been the accumulation in the intima and at times in the media of a mucoid intercellular substance exhibiting the metachromatic staining property of mucopolysaccharides. In our studies we have used the toluidin blue stain to demonstrate this ground substance. Metachromasia is reduced or abolished by treating sections with hyaluronidase, which indicates that the mucoid substance may be a hyaluronate. There is no doubt that the sclerotic arterial lesions are related to the pyridoxine deficiency. Lesions of this character have not been found in other deficient states or in control animals maintained on adequate supplements of pyridoxine. Arterial lesions of pyridoxine deficiency are widespread and the histologic picture in vessels of various sizes corresponds closely to the changes seen in comparable vessels in man.

To what extent is this experimental arteriosclerosis related to the spontaneous disease in man? At present it can be related only by analogy and inference. The morphologic analogies appear substantial. During the past 2 years we have studied the morphologic sequences in the development of human arteriosclerosis, paying particular attention to the changes seen in the coronary arteries. It is our belief that the disease in man is primarily degenerative and proliferative in character, and that in the majority of cases the appearance of lipids and cholesterol represents a secondary phenomenon. Reference to earlier studies of arteriosclerosis and observations in our laboratory substantiate this viewpoint. It is of interest that the early morphologic studies, including Virchow's initial application of the histologic method, support the concept. Aschoff accepted this concept and placed particular emphasis on the thesis of lipid imbibition at the sites of degeneration. Schultz considered a mucoid alteration of the intima as the basic phenomenon of arteriosclerosis and presented substantial evidence for this view. He believed that the mucoid substance had a special affinity for lipids. This is an attractive thesis.

Our own studies may be summarized briefly as follows: In urban American adults some degree of arteriosclerosis is nearly universal. Early lesions are primarily of a degenerative and proliferative character. In the coronary arteries deposition of lipid substances is rarely a primary factor. While the histologic analogy is substantial and it seems highly probable that pyridoxine deficiency in man would cause lesions of the type seen in the monkey, it does not necessarily follow that pyridoxine deficiency is the only or most important mechanism in the pathogenesis of the human disease. Further study will be essential to establish the rôle of pyridoxine deficiency in arteriosclerosis.

*Discussion*

(Dr. Joseph J. Lalich, Madison, Wis.) What were the weights and fluid balances in these animals?

(Dr. Jerome T. Syverton, Minneapolis, Minn.) Along that same line, I should like to learn whether Dr. Rinehart related these changes to the age of the monkey.

(Dr. Russell L. Holman, New Orleans, La.) I would like to inquire as to the normal level of blood cholesterol or other lipid substances in the blood of the monkey. I think we see in certain early human lesions a predominance of lipid material. I should like to hear Dr. Rinehart's comment about the species variation in relation to this lipid factor.

(Dr. Samuel K. Elster, Washington, D.C.) Will Dr. Rinehart say something about the periodicity of the banded fibers in the electron micrograph?

(Dr. P. O'B. Montgomery, Dallas, Texas) I should like to ask if the monkeys were hypertensive, or what Dr. Rinehart's opinion is about hypertension in the development of the lesion.

(Dr. Rinehart) The animals, at the time they were examined, had all lost much weight. They were all in relatively severe states of depletion. The animals had been maintained on deficient diets for 6 months or longer. I believe that in the pyridoxine deficiency state the animal's hydration is below normal; when they are given pyridoxine there seems to be a phase of hemodilution associated with recovery. Although they may appear somewhat edematous around the eyelids, I think they are actually dehydrated.

The animals studied were all immature monkeys; they were under 2 or 3 years of age at the onset of the experiments. We have not had an opportunity to study this problem in older animals.

The level of the blood cholesterol depends somewhat on the diet. In control animals receiving extra cholesterol the plasma values range from 200 to 300 mg. per cent. Values up to 200 mg. per cent may occur even if the animals are maintained on a low fat diet without cholesterol.

I cannot agree with Dr. Holman's comment about the predominance of lipids early in human arteriosclerosis, with the possible exception of that in the aorta; I think that this is a rather important thing to settle. Is the appearance of lipids an early, or a late factor, or even a necessary factor in the evolution of arteriosclerosis?

I am not able to answer Dr. Elster's question regarding the periodicity of the fibers at this time.*

Dr. Montgomery has raised the question regarding hypertension. It is difficult to take the blood pressure in monkeys, although we have been able to determine the systolic pressure with reasonable accuracy. Certain of the deficient animals had a mildly elevated systolic pressure, in the neighborhood of 135 as against 110 mm. of Hg, but this has not been a striking feature of the deficiency.

CARBOHYDRATE SPECIFICITY OF THE PERIODIC ACID-SCHIFF'S REAGENT (PAS) METHOD. J. F. A. McManus, Birmingham, Ala.

*Abstract.* Many materials in tissue sections color with Schiff's reagent after periodic acid oxidation—the PAS method. These include the known carbohydrates, such as glycogen and mucin, and many other materials whose carbohydrate nature was neither known nor suspected, such as basement membrane and reticulin. Proof of the carbohydrate nature of materials coloring by the PAS method is desirable for two reasons: (1) Other materials besides aldehydes can recolor Schiff's reagent (Lison). (2) Besides the 1,2 glycols, characteristic of carbohydrates, alkylamine and amino substituents of 1,2 glycols are oxidized by periodic acid to produce aldehyde, the reaction upon which the PAS method depends.

* Subsequently we have made measurements and find the periodicity to approximate 500 Å, although the material at present available is not adequate for a precise determination.

It is thought that conclusive proof of the specificity of the PAS method for carbohydrates is provided by two sets of studies: enzymatic digestion and reversible chemical alteration of the carbohydrate. Enzymatic digestion has been used for a long time in the study of tissue carbohydrates. Malt diastase removes glycogen from a section of human tissue but leaves the large mass of tissue carbohydrates unaltered. McManus and Saunders have reported the removal from sections of all carbohydrates by crude pectinase preparations, fungal in origin. The same effect is produced by purified polygalacturonidase. The crude pectinase contains also pectinesterase and a true pectinase. Acetone fixation is necessary for the action of the pectic enzymes, the material being handled according to Gomori's directions for the preparation of sections for the acid phosphatase procedure. Pectin esterase increases the amount of carbohydrate, or at least the color, shown by the PAS method, while not changing its situation. The information produced by the action of diastase and the pectic enzyme on tissue is limited by lack of knowledge of the necessary linkage in the substrate as well as in homogeneity of both enzyme and substrate.

Reversible chemical alteration of the carbohydrate in the tissue has been accomplished by acetylation of the section with an acetic anhydride pyridine mixture (McManus and Cason). This procedure attaches acetyl groups to the hydroxyl of the 1,2 glycols as well as to their amino and alkylamine substituents. Periodic acid oxidation no longer produces aldehyde from the acetylated carbohydrates. Weak alkali will release the acetyl groups from the 1,2 glycol in a short time but a longer period is required to saponify the N-acetyl. The procedure of acetylation and de-acetylation allows confirmation of the 1,2 glycol linkage in the materials coloring with Schiff's reagent after periodic acid oxidation, *i.e.*, the carbohydrates of tissues.

The materials whose carbohydrate nature has been confirmed include all those previously reported as positive by the PAS method. The acetylated sections do not color with Schiff's reagent. Lison's worry about acetone and acetyl groups recoloring Schiff's reagent appears unfounded. The combined proof of coloring with Schiff's reagent after periodic acid, failure so to color after acetylation, and return of coloring by the PAS method after alkalinization is sufficient to indicate a carbohydrate in sections. This conclusion further introduces the investigation of the mechanics of the pectic enzyme effects on tissue as well as the dependence of various staining reactions on hydroxyl or amine groups.

### *Discussion*

(Dr. Shields Warren, Boston, Mass.) After seeing the slides that Dr. Rinehart showed in the preceding paper, I wonder whether you have happened to carry through any sections of arteriosclerotic lesions by this means.

(Dr. McManus) Yes, Dr. Warren, we have, but not extensively. There is coloring with the Schiff's reagent after periodic acid in this mucoid material which Dr. Rinehart is speaking of. It is questionable whether it is hyaluronic acid when it dissolved by hyaluronidase because other carbohydrates have been shown to be depolymerized by hyaluronate.

THE EFFECT OF SENSITIZATION AND X-RADIATION ON THE METABOLISM OF $I^{131}$-LABELED PROTEINS. G. J. Dammin, F. J. Dixon, and S. C. Bukantz (all by invitation), St. Louis, Mo.

*Abstract.* $I^{131}$-labeled proteins have been used in this investigation of the pathogenesis of the vascular lesions induced in rabbits by foreign protein. The degree of iodinization has been observed not to affect the antigenicity of the foreign proteins used, bovine gamma globulin (BGG) and bovine serum albumin (BSA). Proteins so labeled are relatively stable under a wide variety of conditions, the liberation of $I^{131}$ requiring either a denaturation or disintegration of the protein molecule.

Labeled homologous gamma globulin (RGG) injected intravenously into normal rabbits exhibited two phases of elimination from the blood: an initial phase with rapid elimination of about 50 per cent in 6 hours, and a phase of slow constant elimination which continued for 14 days. The first, we have termed the "dilution" phase, the second, the "non-immune" phase. This resembles the curve of elimination for injected homologous proteins when such proteins are labeled *in vivo* by feeding $C^{14}$ or $N^{15}$ as labeled lysine. Labeled BGG in normal rabbits was eliminated from the blood in 3 distinct phases: "dilution," "non-immune," and a phase of rapid elimination which continued until the 8th day when less than 0.1 per cent of the injected protein was present. This we have termed the "immune" phase. Serologic studies demonstrated that anti-BGG antibody appears on the 4th day. Sensitized rabbits receiving BGG showed greater elimination from the blood initially, presumably because of the simultaneously acting immune mechanism. An "immune" phase followed which paralleled that of the normal rabbits. Rabbits receiving 500 r. total body irradiation 2 days before injection showed the "dilution" phase, then a "non-immune" phase for about 7 days, and thereafter a more rapid loss. More than 0.1 per cent of the injected protein was still detectable on the 14th day, when antibody appeared. With BSA, the "immune" phase appeared later in normal rabbits and the rate of loss was somewhat slower.

Most of the radioactivity eliminated from the blood could be accounted for in the urine. Antigen was not detectable in the urine serologically, nor was any activity protein-bound. Partition radiograms constantly showed most of the activity in the form of iodide, with smaller portions of diiodotyrosine, and an unidentified fraction. In 6 days sensitized rabbits had excreted 80 per cent of the injected activity; normals, 64 per cent; and x-radiated, 55 per cent. Labeled BGG in mice produced similar results, 60 to 70 per cent of the injected activity being found in the urine in 3 days. There was no particular localization in the spleen, lymph nodes or appendix. In fatal anaphylaxis, autoradiographs showed activity, and therefore antigen, to be concentrated in acellular accumulations in focal dilatations of the pulmonary capillaries. The results differ from those obtained when dye is used to label BGG, the dye-BGG complex being handled more like a particulate antigen.

The use of $I^{131}$ as a protein label permits accurate study of the metabolism of homologous and heterologous proteins in the rabbit. Rates of elimination from the blood may be useful as an index of immunity or of exposure to ionizing radiation and other agents affecting antibody production.

*Discussion*

(Dr. Chester R. McLean, Montreal, Que.) Did any of the experimental animals live for a sufficiently long period to develop generalized lesions of serum sickness; and, if so, was there any increase in the concentration of radioactive iodine in these organs? Did the thyroid metabolism of these animals interfere with the excretion of radioactive iodine?

(Dr. Israel Davidsohn, Chicago, Ill.) Can you suggest the reason for the difference in the amount of antigen found in the lungs and heart as compared with the spleen and blood? Is it possible that local formation of antibodies in these tissues plays a part; for example, that the larger amount of antibodies produced in the spleen may account for less antigen being found there and the smaller amount produced in the heart for larger amounts of the antigen remaining?

(Dr. Jerome T. Syverton, Minneapolis, Minn.) I should like to learn the factors of x-radiation, that is, the dose and the radiation factors, and whether the excretion of normal homologous protein and foreign protein were similar.

(Dr. B. Black-Schaffer, Durham, N.C.) Was the presence of radioactivity in the tissues several days after injection due to the presence of the iodine-globulin complex, or to radioactive iodine dissociated from the globulin? Were serologic tests carried out with extracts of the tissues for that purpose?

(Dr. Dammin) The doses of the bovine gamma globulin used were much smaller than those usually required to produce lesions, 75 and 500 mg. Hawn and Janeway obtained glomerular lesions only when they used as much as a gram per kilo of bovine gamma globulin. We did not observe glomerular or other lesions such as have been observed after larger doses of bovine gamma globulin. All animals, rabbits and mice, were pre-treated with iodine so that there would be less uptake of iodine by the thyroid and at no time was more than 1 per cent of the total activity found in the thyroid.

With reference to the localization of the antigen in the lungs and heart, we have not yet studied the antibody content of tissues to determine to what degree its presence can account for antigen localization. As to what happens in the anaphylactic animal, you may recall the slide which showed the striking uptake of the antigen by the lung and the slide showing radioactivity, and therefore antigen, localized in the pulmonary capillary dilatations with none in the other portions of the capillaries and smaller arteries. This suggests that there may be tissue as well as humoral antibody involved in this phenomenon.

The x-irradiation given to the rabbits consisted of 500 r. units given as total body irradiation.

The homologous protein was metabolized and eliminated slowly, and in two phases, "dilution" and "non-immune," resembling somewhat the curve shown by the x-irradiated rabbits.

We were concerned initially with whether we were measuring originally-labeled protein when we measured the protein-bound activity. From the evidence quoted, we felt that this was so. We intend to investigate this aspect further by testing for antigen in tissues no longer showing radioactivity. Our studies on the blood show that when we can no longer measure activity in the blood, we are unable to detect any of the originally-labeled protein by serologic means.

INTRACELLULAR REACTION TO PARTICULATE ANTIGEN. Howard C. Hopps, Oklahoma City, Okla.

*Abstract.* Fine particulate matter, carbon, talc, silica and aluminum hydroxide, to which horse serum was adsorbed, was injected intradermally into rabbits. Following its ingestion by macrophages, histopathologic studies were made at various intervals to determine the nature of degenerative and proliferative changes. Cellular reaction varied considerably but in many instances amounted to massive necrosis and at certain periods was characterized by tuberculoid lesions with proliferating macrophages arranged about areas of caseous-like necrosis. Selective stains for reticulum and collagen revealed that in the presence of antigen-antibody reaction, these substances were not formed as readily as in control lesions.

*Discussion*

(Dr. Alan R. Moritz, Cleveland, Ohio) Were these observations made on animals that were sensitized to this antigen? I may have missed that in the presentation.

(Dr. Benjamin Highman, Bethesda, Md.) What was the reaction to silicon dioxide?

(Dr. Hopps) Dr. Moritz, these normal animals had not been sensitized to horse serum. Apparently the sensitization occurred in the course of the experiment. We have some experiments under way to determine the reaction of animals previously sensitized.

The reaction to silicon dioxide was not represented because there was such a reaction even to the non-adsorbed silicon dioxide that it obscured any differences that might have been present. With the other materials there was a difference in reaction to coated and uncoated particles as early as 1 week (the shortest time at which tissue reaction was observed) and this persisted through the 8 weeks' period.

DIFFUSE GLOMERULONEPHRITIS INDUCED IN RABBITS BY REPEATED SMALL INTRAVENOUS INJECTIONS OF WHOLE HORSE SERUM. Chester R. McLean (by invitation), John Fitzgerald, Omar Younghusband (by invitation), and John D. Hamilton, Kingston, Ont.

*Abstract.* This report is concerned with lesions of diffuse glomerulonephritis induced in rabbits by small daily injections of horse serum administered over varying periods. Thirty-one albino rabbits were given a daily intravenous injection of 0.5 cc. of horse serum. The animals were divided into four groups and sacrificed at progressively longer intervals ranging from 3 to 13 months. Unilateral nephrectomy was carried out at some time during treatment on 20 of the 31 treated animals. Seven animals died during the course of the experiment or were killed because they appeared critically ill. In all of these cases the animals had been treated for a sufficiently long period to provide significant material for histological study.

Twenty of the 31 treated rabbits (64.5 per cent) exhibited diffuse glomerulonephritis. In their morphologic appearances, the experimentally induced lesions bore a marked resemblance to those of human glomerulonephritis. In 14 cases the renal changes were classified as acute proliferative glomerulonephritis and in the remaining 6 cases as advanced subacute and chronic glomerulonephritis. The earliest detectable changes in the acute proliferative group appeared to involve simultaneously both endothelial and epithelial cells, and to a lesser degree, the basement membranes of the capillary loops. The affected glomeruli appeared somewhat larger than normal. Swelling and proliferation of endothelial cells led to partial or complete obstruction within one or several capillary loops of the glomerulus. Proliferation of the glomerular and capsular epithelium with the formation of epithelial crescents appeared to occur coincidentally with the endothelial changes. In the severest degrees of this process, the glomerulus appeared to be wholly disrupted. The outlines of the entire glomerular tuft became replaced by a syncytial mass of swollen cells of mixed endothelial and epithelial origin.

The renal lesions which were classified as subacute and chronic glomerulonephritis were of severe degree. Collagen fibers appeared between the adjacent capillary loops and in Bowman's capsule, so that adjacent capillary loops became adherent to Bowman's capsule and to one another. Progressive fibrosis within and around the glomerulus led to complete disorganization and obliteration of the capillary tufts within that structure. In such instances, complete atrophy and involution of the associated tubules, or marked dilatation with flattening of the lining epithelium was the rule. The changes of tubular atrophy, dilatation, and cast formation, when found, were seen always in association with severe glomerular damage. The renal lesions were progressive in that they passed through acute, subacute, and chronic phases with eventual scarring, atrophy, renal failure, and blood urea retention to levels which in the human are regarded as uremic.

In numerous experiments it has been well established that the intravenous injection of one or more large doses of horse serum will produce in the rabbit focal inflammatory lesions of the heart valves and visceral arteries, which resemble in many respects the lesions of human rheumatic fever and periarteritis nodosa respectively. In view of this fact, we consider it to be especially noteworthy that diffuse glomerulonephritis was the only significant lesion produced under the conditions of this experiment. This distinctly different morphologic picture, in which nephritis has occurred in the absence of arteritis and cardiac valvulitis, appears to be directly related to the method of administering the antigen, that is, the injection of small daily doses of serum over long periods of time.

*Discussion*

(Dr. Howard C. Hopps, Oklahoma City, Okla.) I should like to ask whether there were changes in the juxtaglomerular apparatus, and whether the blood pressure was measured, and if so, what the levels were.

(Dr. B. Black-Schaffer, Durham, N.C.) What was the shortest interval from the beginning of the injections to the appearance of clear-cut glomerular lesions?

(Dr. Otto Saphir, Chicago, Ill.) Most of the animals showed proliferative changes in the glomeruli. I wonder whether other glomeruli showed the exudative changes which we see so often in man in typical acute hemorrhagic glomerulonephritis.

(Dr. L. L. Waters, New Haven, Conn.) Were any determinations made to see whether these animals had become sensitive to the horse serum during the course of injections?

(Dr. McLean) In reply to Dr. Hopps, the blood pressures were not recorded in any of the animals of this pilot experiment, and studies of the juxtaglomerular apparatus were not performed.

In response to Dr. Black-Schaffer, 3 months was the shortest period of serotherapy in which glomerulonephritis was observed. Severe renal lesions were found in one animal killed accidentally during left nephrectomy. We feel that as the duration of treatment is prolonged, more of the animals in any given group will develop nephritis, although it may appear in isolated animals within a relatively short period.

We did note, Dr. Saphir, that exudative changes in the glomeruli were not conspicuous, and in this respect these experimentally induced lesions appear to differ from some types of the human disease. We never saw free hemorrhage into the capsular space, and polymorphonuclear leukocytes were not numerous.

With respect to Dr. Waters' question, the animals tolerated the daily injections very well. Severe anaphylactic shock was not encountered and none of the animals in this series died of it. Twenty-nine of the 31 treated animals developed serum precipitins and positive skin tests within 3 weeks of the introduction of serum therapy. Their titers rapidly climbed to 1:1000 or 1:2000 and remained relatively constant throughout the experiment. It is remarkable that 2 animals were treated for 13 months and failed to develop demonstrable antibodies or a positive skin test.

HYPERTENSION IN RATS FOLLOWING COMPLETE RENAL ISCHEMIA. Simon Koletsky, Cleveland, Ohio.

*Abstract.* Complete ischemia of one rat kidney for a period of 2 or 3 hours, followed by resection of the opposite kidney, leads to the development of sustained hypertension. The latter is associated with some degree of chronic renal insufficiency. The ischemic kidney undergoes compensatory hypertrophy, but this is followed by the development of a progressive lesion of glomerulonephritic type terminating in renal decompensation and uremia. Autopsy reveals cardiac hypertrophy and generalized vascular disease.

*Discussion*

(Dr. Emmerich von Haam, Columbus, Ohio) Was the time, 3 hours, the minimum period or the optimum period for the development of hypertension?

(Dr. James F. Rinehart, San Francisco, Calif.) I did not get clearly the total duration of the experiments. What were the changes in the injured kidney if the opposite kidney was not removed?

(Dr. Bruce Taylor, Chicago, Ill.) Was hypertension produced in any other experimental animal by this method?

(Dr. G. Lyman Duff, Montreal, Que.) I think the inflammatory lesions in the arteries that Dr. Koletsky illustrated are very similar in appearance to those that were reported some years ago as developing spontaneously in untreated rats between 600 and 900 days of age by Wilens and Sproul. The lesions are similar also to those reported by Ham following a single massive injection of irradiated ergosterol, and then a lapse of a period of months. Similar lesions were reported by Hall following repeated doses of acetylcholine, and by Selye and Pentz following injections of desoxycorticosterone acetate. I should like to ask if the lesions Dr. Koletsky described differ in any way from those produced in rats under these varying conditions.

(Dr. William H. Carnes, Baltimore, Md.) What happens to the vessels that have been clamped in the ensuing time, and what are the initial changes in the ischemic kidney, or the early changes within a matter of hours or a few days following the experimental procedure?

(Dr. Otto Saphir, Chicago, Ill.) To me the most interesting observation is that hypertension did not ensue with the production of ischemia in one kidney, but that hypertension occurred when the opposite kidney was removed. I wonder if the other normal kidney does not contain something that prevents the development of hypertension, and that only by its removal is the inhibitory factor removed and hypertension follows. I should like to hear whether this is your explanation, Dr. Koletsky.

(Dr. Koletsky) In answer to Dr. von Haam, the 3-hour period is the optimum one and gives the maximum number of animals with sustained chronic hypertension. The experiments as we set them up included rats which underwent this procedure at periods from 30 minutes to 5 hours.

In answer to Dr. Rinehart, who raised the question of changes in the injured kidney if the opposite one is not removed, we have studied the anatomical changes that follow cessation of blood flow to one kidney. In these experiments we placed a clamp across the renal hilum for 2 or 3 hours, removed it, and then sacrificed the animals at intervals which ranged up to 1 year. We also determined the blood pressure in these experiments. The clamped kidney undergoes a marked degree of diffuse tubular necrosis without distinct changes in the glomeruli or blood vessels. The tubular necrosis is followed by complete repair, but this is accompanied by very marked shrinkage of the organ. The organ literally melts down to one-fifth of its original size. It will stay in that condition as long as the opposite kidney is intact, but once the opposite kidney is removed, as determined in another set of experiments, the tiny kidney will undergo marked hypertrophy and will very rapidly increase to about its original weight or more. In total duration the experiments ranged from a few weeks to about 1 year.

Dr. Taylor asked about the use of other animals. We employed only rats. However, Dr. Orbison in our department is now engaged in a preliminary experiment using rabbits, and is utilizing this technic.

Dr. Duff remarked on the occurrence of similar vascular lesions in rats following injection of certain substances. I would be inclined to concur with this observation.

Dr. Carnes raised the question of injury to the clamped kidney. Tubular necrosis develops rapidly and is followed by repair and renal atrophy. As long as the opposite kidney remains intact, compensatory hypertrophy does not occur. Aside from possible medial thickening, the blood vessels do not appear to be significantly altered.

I agree with Dr. Saphir that the mechanism of hypertension in these experiments is related to the presence of intact or normal-functioning renal tissue. We have not observed the development of high blood pressure in rats which had one intact kidney. However, following its removal the blood pressure went up, so it would appear that the normal kidney exercised some mechanism in the maintenance of normal pressure.

ACUTE NON-STREPTOCOCCAL GLOMERULONEPHRITIS—EXPERIMENTAL DEMONSTRATION OF A PATHOGENESIS. B. Black-Schaffer and (by invitation) S. B. Silverman, Durham, N.C.

*Abstract.* It has been shown * that a characteristic Shwartzman phenomenon may be elicited in the hypersensitized rabbit if the appropriate antigen plus a Shwartzman-preparatory bacterial toxin are inoculated together intradermally, the local antigen-antibody reaction provoking the phenomenon. By this means, material

* Black-Schaffer, B., Milam, J. W., Brockman, D. D., Coonrad, E. V., and Silverman, S. B. Production of the Shwartzman phenomenon by a single injection technic. *J. Exper. Med.*, 1950, **91**, 539–548.

amplification of the allergic reaction may be obtained. This principle of amplification was applied systematically by inoculating hypersensitized rabbits intravenously with antigen (1.0 to 3.0 cc. of 5.0 per cent bovine gamma globulin) mixed with bacterial toxin. In a small number of animals (15 of 25) acute glomerulonephritis was observed within 24 hours following the inoculation. In 2 of the 15 it was extremely severe. Hypersensitized control rabbits, 29, receiving antigen or toxin alone, or both separated by at least 5 hours, did not reveal a renal lesion. It is suggested that the inconspicuous results of antigen-antibody reaction in the glomeruli, at the low dose level of antigen used, are amplified, as in the skin, by provoking the Schwartzman-prepared glomerular capillaries, producing glomerulonephritis. This mechanism is thought to be applicable to certain cases of non-streptococcal glomerulonephritis illustrated by the following: A man with a history of sulfonamide hypersensitivity, during the course of a resistant necrotizing staphylococcal maxillary and ethmoid sinusitis suffered a fatal systemic reaction to sulfathiazole, marked by arthritis, fever, progressive oliguria, and uremia. The kidneys revealed fulminating glomerulonephritis. It is proposed that this was prepared by staphylococcal products, and provoked by the antigenic form of the drug reacting with its antibody in the glomeruli. The phenomenon is interpreted as a local Shwartzman reaction of the kidney which materializes, because of the presence of bacterial products, an otherwise negligible antigen-antibody reaction.

*Discussion*

(Dr. Chester R. McLean, Montreal, Que.) I would like to ask Dr. Black-Schaffer if he would comment on the similarities or dissimilarities between the necrotizing renal lesions which he has produced, and those produced by diphtheria toxin. Did this experimental procedure induce lesions in the renal tubules or in any organs other than the kidney?

(Dr. Black-Schaffer) We did not utilize diphtheria toxin. That work was done by Ahlström, one of whose papers I discovered a few weeks ago. He did an experiment similar to ours, using staphylococcal toxin in amounts less than necessary to produce cortical necrosis of the kidney and horse serum. It is interesting that our bacterial toxin may likewise produce cortical necrosis of the kidney, but only when given in two intravenous injections several hours apart. In this respect the two bacterial toxins have common properties. Ahlström utilized diphtheria toxin as well as cobra venom and Dick toxin with negative results. The lesion in these animals is not at all similar to that produced by staphylococcal toxin or by two injections of our bacterial toxin. There was no suggestion of cortical necrosis of the kidney; the lesion produced is a glomerulonephritis; the glomeruli were involved as well as the tubules.

Concerning the second question, we have not studied all the organs of all of our cases. We, however, looked at a sufficient number to feel confident that there is little to be seen outside of an occasional example of centrolobular liver necrosis. This lesion may be produced by the toxin alone.

CHANGES IN THE CORONARY AND VISCERAL ARTERIOLES OF DOGS FOLLOWING LARGE DOSES OF ADRENALIN. L. L. Waters (by invitation), New Haven, Conn.

*Abstract.* Although the association of hypertension and arteriolar disease is supported by a wealth of information derived from the clinical and pathologic study of hypertensive patients, there has been little direct experimental evidence that high blood pressure *per se* results in acute or chronic arteriolar lesions. The experiments to be presented bear on this subject.

Following the observation that injections of a pressor substance, N-amylamine, into dogs result in segmental necrosis of the coronary arterioles, the vascular-damaging effect of large intravenous injections of adrenalin was re-investigated.

Since animals developing arteriolar lesions after N-amylamine had maintained mean arterial blood pressures in the range 220 to 280 mm. of Hg for 30 minutes following each injection, enough adrenalin in divided doses was injected intravenously into normal dogs to duplicate the magnitude and duration of this rise. (Four to 8 cc. of 1:1000 solution in doses of 1 cc. each usually were required.) This procedure was carried out on each of 3 days. At appropriate intervals thereafter (18 hours to 10 days) the animals were sacrificed and their tissues examined. Hemorrhage and necrosis of the media of the thoracic aorta were present frequently. Of greater interest was the consistent finding of segmental necrosis and hemorrhage of the small muscular coronary arteries, and of similar arteries in the stomach, gall bladder, and large intestine. Although in some instances focal medial necrosis and hemorrhage were found in the intralobular and arcuate renal vessels, the kidneys were not regularly involved. In none of the animals was the non-protein nitrogen of the blood elevated. Microscopically, the arterial lesions varied from fibrinoid necrosis of the media to an intense panarteritis with abundant perivascular cellular exudate. The lesions went on to intimal thickening, fibrosis of the media, and perivascular scar. Besides the vascular lesions, focal hemorrhages and necroses of the myocardium often were extensive. Pre-treatment of a series of dogs with amounts of dibenamine that abolished the blood pressure rise due to the subsequent injections of adrenalin, prevented completely the arterial and myocardial lesions.

*Discussion*

(Dr. Chester R. McLean, Montreal, Que.) Byrom and Dodson, in 1948, reported the induction of acute arterial necrosis in rats subsequent to repeated short episodes of severe hypertension produced by the direct, rapid injection of a few cubic centimeters of Ringer's solution into the aorta. In their material the vascular lesions were particularly severe in the kidneys, and in this respect differed somewhat from the lesions noted here. I wonder if Dr. Waters would care to remark on how comparable his results are to those of Byrom and Dodson, and whether or not he attributes his lesions to the hypertension *per se*. I would also like to know if he saw collagen necrosis in the vascular lesions.

(Dr. Paul Klemperer, New York, N.Y.) With the authorization of Dr. William Ehrich I should like to refer to his observation which I think is very much in line with the experiments of Dr. Waters. This concerns a colored girl who died at the age of 19, after having had attacks of paroxysmal hypertension for 2 years. At autopsy a pheochromocytoma was found in one of the adrenals. In various organs, particularly striking in the intestine and also in the kidney, there were necrotizing vascular lesions even more severe than those which Dr. Waters has shown. I believe the mechanism by which Dr. Waters produced these necrotizing lesions may have some bearing also in human pathology. I could not find any similar lesions in our cases of pheochromocytoma, but most of our cases were of the older age group.

(Dr. Jacob Werne, Jamaica, N.Y.) I wonder whether any of these animals were observed to go into shock or die in shock. The doses of adrenalin tolerated seem to be especially large. An experience occurring in my routine medicolegal practice is recalled in which an individual died instantaneously following the accidental intramuscular injection of about 8 cc. of adrenalin. It would be interesting to know whether any of these dogs showed intravascular thrombi.

(Dr. Peter Gruenwald, Brooklyn, N.Y.) In asphyxiated newborn infants lesions similar to those shown here are found in the coronary and hepatic arteries. These infants may be stillborn or live up to 3 days of age. They certainly do not have an increased arterial pressure, but possibly an increased venous pressure. I would appreciate it if Dr. Waters could suggest an explanation for this.

(Dr. Conrad L. Pirani, Chicago, Ill.) I would like to ask Dr. Waters how he

explains the fact that in some organs there are considerably more severe lesions than in others. I cannot see how the increase in venous pressure which he has mentioned would cause these arterial lesions.

(Dr. Waters) We have not yet been able to repeat Byrom and Dodson's work. In their experiments the kidneys are rendered ischemic, or nearly so, for some minutes after the forcible injection, and it might be that anoxia as well as hypertension is a factor.

Thank you, Dr. Klemperer.

In reply to the question about the animals dying in shock, some did die, about 4 or 5 out of the series of 18 animals that we have done, and as nearly as we can tell they died in ventricular fibrillation. There was no evidence of marked pulmonary edema. We did not find any intravascular clotting, although it was looked for.

I did not know that infants dying of asphyxia have necrotizing lesions in their vessels. I should like to look that up.

Lastly, Dr. Pirani asked why lesions are more severe in some organs than in others. I have no explanation for that.

ASYMPTOMATIC FOCAL ARTERITIS; 88 CASES.* Alfred Plaut, Topeka, Kans.

*Abstract.* Single, sparse, or numerous arteritic foci of "periarteritis nodosa type" were found in 88 of 6,576 appendices. Most foci were not larger than one-third of a mm., some reached three-fourths of a mm., one measured 1.2 by 1.0 by 0.8 mm. The nodules showed all imaginable variations in the ratios of necrosis, subendothelial deposits, neutrophilic and eosinophilic leukocytes, plasma cells, and adventitial cells. The outer layers of many of the nodules were edematous. No aneurysms were found. The lesions often occupied a bifurcation. Highly cellular foci with severe necrosis obviously represented young lesions; smaller, more compact nodules with many spindle cells were interpreted as older ones. There were no characteristic scars. Arteries of all layers of the appendix were affected; the distribution was entirely irregular. The tip was a preferred site, the arteritis was found in obliterated and in non-obliterated portions. The lesion might be severe in one block and absent in a neighboring one. There was no relation to neuroma and to carcinoid. Occasionally the mesoappendix contained foci. Old and young lesions occurred near each other. Sometimes a single definite lesion was accompanied by what seemed to be "formes frustes." There was no relation to a clinical picture. Inflamed appendices showed the lesion, prophylactically removed appendices did, and appendices from unselected autopsies also. Massive lesions were more numerous in young people. No relation to a drug was established. The lesion was more frequent in gynecologic material. The peak for males was in the third decade; for females, in the second. In girls 15 to 19 years of age the arteritis seemed to be more frequent than from 10 to 14; no such difference existed for boys. Pregnancy had no influence upon the occurrence of focal arteritis.

The lesion must be extremely rare in other organs. It was seen once in the ligamentum latum, once in the hilar stump some years after nephrectomy, once in kraurosis vulvae, once in a fibrotic partly ossified fallopian tube, and twice in the ovary.

The genesis of this little known form of arteritis is obscure. This reactive abnormality of blood vessels is restricted to a small area; we must, therefore, think of local factors. Familiarity with this lesion will prevent unwarranted alarming diagnoses.

*Discussion*

(Dr. Russel L. Holman, New Orleans, La.) Have you made any attempt to correlate these lesions with recent changes in body weight, Dr. Plaut? The results

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

might conceivably be related to a high fat or variable diet with or without hormonal influences. I think we are all aware that there may be rather dramatic changes in body weight during puberty or adolescence.

(Dr. Plaut) No special study of diet was made, but I think I am justified in saying that, with the large number of cases examined, and with the lesions occurring in so many young healthy people, there cannot have been marked changes in weight.

STUDIES ON THE PATHOGENESIS OF NECROTIZING ARTERITIS. Russell L. Holman, New Orleans, La.

*Abstract.* The sequence of feeding a "standard high fat diet" for 8 weeks or longer and then producing "standard renal insufficiency" has been shown previously to induce "typical arterial lesions" in mongrel dogs regardless of sex and age. Either factor alone has proved to be ineffective. The pathogenesis of these arterial lesions, which closely resemble those of rheumatic arteritis and periarteritis nodosa, is obscure, but is being studied from the standpoints of a "dietary factor" and a "renal factor." Various fatty substances have been bioassayed for the "dietary factor" by keeping the "standard diet" isocaloric and varying only the fatty substance. The following fatty substances have yielded negative results: corn oil, lard, olive oil, cocoanut oil, mutton tallow, and oleomargarine. Positive results have been obtained with certain samples of cod liver oil (in 36 of 40 dogs) and with creamery butter (in 8 of 8 dogs). Studies to date indicate that the "renal factor" is an, as yet, undetermined metabolic product of renal tubular epithelium. Conjectures on the pathogenesis of these "typical arterial lesions" are presented in the light of various "conditioning factors" (vitamin E, cholesterol, reversibility, variation in quantity and quality of protein) and an attempt is made to correlate these studies with other experimental studies and with similar lesions in man.

*Discussion*

(Dr. Alfred Plaut, Topeka, Kans.) Dr. Holman has shown in his previous work that such lesions can be prevented by vitamin E. Can he tell us what he thinks of the relation of vitamin E to these lesions?

(Dr. John W. Harman, Madison, Wis.) I would like to point out that the clearly distinct difference between the two diets is the absence of vitamin E in the lard diet and its presence in the diets containing corn oil and the other vegetable oils. I wonder if, in view of Paul György's work and other work on necrotizing lesions which depend on the deprivation of vitamin E, this whole concept might not fit into a metabolic disturbance, as Dr. Holman suggests, rather than bear a relation to antigen-antibody reactions. Certainly there is a curious relationship to the necrosis of various tissues, particularly the kidneys and liver in the vitamin E animal which is given alloxan, and this probably is pertinent to this particular work.

(Dr. Holman) We have been much interested in the question of vitamin E in these animals as we have previously shown that vitamin E will prevent the lesions after feeding cod liver oil. When we found that butter contains the "dietary factor," we tested the effect of vitamin E in preventing the arterial lesions after feeding butter for 8 weeks or longer. It will definitely prevent the lesions. We now have some experiments in progress to determine whether vitamin E therapy will prove effective at various intervals after the production of renal insufficiency. We cannot report on these experiments yet, but we can report that up to 72 hours after the induction of "standard renal insufficiency" in the case of cod liver oil-fed dogs, vitamin E has a protective action. The obviously low vitamin E content of the "positive" diets may be a fundamental factor in this work. The way in which vitamin E may act intrigues us in trying to interpret these lesions. Thus far we have been unable to find any definitely consistent changes in the plasma vitamin E level, in the urine, in the blood pressure, in the electrocardiogram, or in any of the blood plasma lipids,

by which we might predict the presence or absence of lesions in these animals. We still have to sacrifice the animals to see if they have lesions. Presumably vitamin E prevents the "oxidation" of one or more unsaturated fatty acids that pile up in the body during the 8 weeks or more of specified high fat feeding and reach "explosive levels" after the induction of renal insufficiency. I do not know what the answer is; we have not found it yet.

ISOLATION AND ANALYSIS OF TISSUE STRUCTURE BY PHYSIOCHEMICAL METHODS. George M. Hass, Chicago, Ill. *

*Abstract.* Methods of classical chemistry largely describe materials in the soluble or crystalline state. Hence, they can seldom be used directly to describe components of integrated tissue, for much of tissue is insoluble and non-crystalline. It is possible, however, to investigate tissue structure with a view to developing a physicochemical definition of its properties and composition. The extent to which a tissue component may be altered by the methods employed must be kept in mind and efforts made to relate the observed product to the natural component. Many methods can be carried out on fresh tissue so prepared that a microscopic image of the effect of a chemical manipulation can be detected, if such an image appears. One method of approach is by determination of sharp solubility end-points of tissue components. For most purposes, aqueous buffer solutions with a wide range of pH, ionic strength, and ionic composition are very useful as solvents. Bacterial contamination should be guarded against because this often interferes with interpretation of effects of buffer solutions. Tissue enzymatic action may be equally confusing, because a buffer solution may promote rapid action of natural tissue enzymes. If any end-point for solubility of a tissue component can be found, it may be useful in obtaining primary extraction of the component in relatively pure form. This is important because it is often easier to separate solids by differential solubility from the insoluble state than by fractionation from the soluble state. Furthermore, it is advantageous to be able to associate the exclusive disappearance of a tissue component at a solubility end-point with the simultaneous appearance of substances in the solvent. If a tissue component is soluble, classical methods of chemistry can be applied in study of the soluble product. As properties of the product in solution are defined, they may be used in determining whether this product is closely related to the natural tissue component. This is often a difficult problem which involves reconversion of the soluble product to a form which possesses properties of the natural component. If this can be done, proof of identity is good. Even though proof of identity may be doubtful, significant fractions of the often complex tissue component may become available for study. Other methods may be used when tissue components are insoluble or unduly modified by conditions used to dissolve them. These methods involve physicochemical description of tissue components in as near their natural insoluble state as possible. At times, physical methods of optical, electronic, or mechanical type are useful. At other times, it would seem that a new form of chemistry is needed. The determination of the differential reaction of tissue components to different enzymes is often useful and deserves wider application. Purification or even isolation of intact structural elements may be obtained in this way. The use of specific dyes as chemical rather than optical aids for studying insoluble tissue components is an undeveloped type of analysis. The use of chemical reagents for definition of functional chemical groups in insoluble tissue components has been likewise neglected. The determination of special chemical reactivities or physical properties of a tissue component, exposed to the action of specific organic reagents before and after dissolving the component, deserves further study. Attempts to crystallize tissue components *in situ* by use of reagents, especially suitable for

* Presented by C. Bruce Taylor, Chicago, Ill.

forming crystallizable complexes, have seldom been made. If established methods of physics and chemistry are properly applied and if original physicochemical methods, specially designed for analysis of tissue structure in the natural insoluble state, are developed, a more intimate knowledge of the relations between structure and function will certainly be gained.

*Discussion*

(Dr. Russell L. Holman, New Orleans, La.) I gather from your presentation that you injected into the subcutaneum a lipid substance or a fatty acid. I would like to ask just what fatty acid or what lipid compound you used?

(Dr. C. Bruce Taylor, Chicago, Ill.) I am substituting for Dr. Hass, who, as I understand it, used unsaturated oils and various unsaturated fatty acids from animal and vegetable sources. Synthetic esters of the unsaturated acids were injected.

(Dr. Holman) Was vitamin E used in connection with them?

(Dr. Taylor) No.

(Dr. Jerome Gross, Cambridge, Mass.) How did you remove collagen from the aorta?

(Dr. Taylor) Dr. Hass reported this work in the Archives of Pathology in 1942 or 1943. He used formic acid, 88 per cent. It was done at 40° C. for 48 to 96 hours.

A Summary for the Defense of the Altered Immunity Mechanism in the Collagen Diseases. Ernest Aegerter and (by invitation) Joan Long, Philadelphia, Pa.

*Abstract.* Mesenchymal tissue, the primitive defense organ, is differentiated in post-natal life into reticulum, connective tissue, bone, cartilage, and other tissues. In addition to their supportive and binding functions, these tissues inherit, to a measure, the function of defense and are involved in the immune reaction. It is postulated that altered sensitivity is an expression of perverted metabolism on the part of these tissues resulting in non-specific or ineffectual humeral globulins and in altered, fixed, intercellular, collagenous substances which provide the stage for antigen-antibody clash.

The suggestive evidence that the metabolic alteration is related to the immunity process in the collagen diseases is: (1) the site of the lesion, (2) the morphologic characteristics of the lesions, (3) the experimental evidence that hypersensitivity reactions can produce such morphologic lesions, and (4) the clinical evidences of sensitivity in these diseases. However, the collagen diseases, as represented by serum sickness, periarteritis nodosa, rheumatic fever, rheumatoid arthritis, and lupus erythematosus, are not clinically identical. Indeed, the last two show less clinical evidence of hypersensitivity than do the others, but a loss of the ability to produce immunity. It is postulated that these clinical differences are due to modifications of the immunity reaction by other factors. Among the factors capable of modifying the function of the mesenchymal defense unit are the activities of the endocrine glands. Particular attention has been focused lately on the influence of the pituitary-adrenal axis and its separate parts on the collagen diseases. The action of the adrenal cortical hormone on phagocytosis, antibody formation, and permeability of cell membranes and the ground substance is discussed. The rôle of hyaluronic acid and hyaluronidase in the latter reaction is considered.

The Relation of the Lesions of Hypersensitivity to the Collagen Diseases. Robert H. More, Montreal, Que.

*Abstract.* The term "collagen diseases" has come to include several clinicopathologic entities, the more important of which are rheumatic fever, diffuse scleroderma, disseminated lupus erythematosus, and periarteritis nodosa. The lesions characteristic of these diseases have the common denominator of collagen damage, and tissue hypersensitivity is widely believed to be their exciting cause. However,

as applied to these entities, the term "collagen diseases" may be somewhat misleading. It tends to imply that the morphologic alteration of greatest significance and highest specificity is collagen damage. In so doing it detracts from the significance of at least one other morphologic alteration, which may be of equal importance. This consists of an inflammatory reaction that is primarily and predominantly a large mononuclear cellular response. To illustrate the relation of this total picture of collagen damage and mononuclear inflammatory response in the collagen diseases to the lesions of hypersensitivity, a group of lesions are presented which have been selected from human subjects and experimental animals, in which specific hypersensitivity has been demonstrated. These lesions have as their most characteristic feature, a focal inflammatory response dominated by large mononuclear cells that frequently progress to granuloma formation.

Twenty-three cases, exhibiting lesions attributed to sulfonamide sensitivity, were found at autopsy to have granulomatous lesions in the heart (7 cases), liver (8 cases), spleen (6 cases), kidney (1 case), and coronary artery (1 case). The inflammatory reaction consisted of a relatively dense accumulation of mononuclear cells, diffuse in the heart, or more focal in the liver. The cells had large round or oval nuclei and an abundant eosinophilic cytoplasm. Cell borders were indistinct. This reaction was associated with a loss of parenchyma, edema of adjacent connective tissue, and occasionally with fibrinoid necrosis of vessel walls. In spite of the acute nature of the tissue destruction, the cellular exudate remained conspicuously mononuclear. Basically similar inflammatory lesions in the visceral arteries, heart valves, lungs, and joints have been observed among 250 rabbits rendered hypersensitive with foreign serum protein. Large mononuclear cells were the most common cell type observed in these inflammatory foci, and this in the presence of tissue destruction which ranged from severe fibrinoid degeneration to simple edematous swelling of collagen. These large mononuclear cells possessed a round or oval nucleus and abundant eosinophilic cytoplasm with well defined borders.

The lesions of the collagen diseases and those of known hypersensitivity in man and animals are not morphologically identical. On the other hand, there are morphologic similarities between these groups. It is suggested that the mononuclear reaction of the diseases of known hypersensitivity is also characteristic of the collagen diseases. This similarity suggests that these entities have a common pathogenesis. It therefore seems desirable, in defining the morphologic reactions of the collagen diseases, to include this characteristic mononuclear response. In so doing they fall more closely into line with the tissue alterations of known hypersensitivity. Duff has taken the view that the collagen diseases represent reactions of the connective tissue as a tissue with all of its components participating, while Aegerter and Long go further and refer to them as reactions of the mesenchymal defense system. The latter concept would account for the injury of the supporting tissues and the characteristic mononuclear cellular response seen in the group of diseases commonly accepted as collagen diseases and in the lesions of tissue hypersensitivity in man and in experimental animals. If we are correct in concluding that there are basic similarities in these morphologic responses, we are at best only nearer to an understanding of the pathogenesis of the collagen diseases. Despite this, there still remains the identification of specific factors concerned in their etiology.

*Discussion*

(Dr. G. J. Dammin, St. Louis, Mo.) I should like to ask whether Dr. More has ever observed fibrinoid alteration of collagen in the valves in the experimental animals. I have found this change to be lacking in animals. This seems to distinguish further the valvular lesions in experimental animals from those in rheumatic fever and in lupus erythematosus.

(Dr. More) We have seen fibrinoid necrosis in the cardiac valves of rabbits sensitized to massive injections of horse serum in only 3 rabbits of some 250 so

treated. But we do see in all these cases widespread collagen injury of cardiac valves not progressing to fibrinoid necrosis. In my opening comment I pointed out that Klinge believed that widespread collagen damage, including fibrinoid necrosis, was characteristic of the diseases under discussion, and I believe *widespread* collagen damage is the important central point to make with reference to alteration of collagen in the collagen diseases and the lesions of known hypersensitivity.

BLOOD FACTOR IN ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS. John R. Haserick (by invitation), Cleveland, Ohio.

*Abstract.* The technic is outlined for demonstrating the factor present in the plasma of patients ill with acute or subacute disseminated lupus erythematosus which induces a positive lupus erythematosus test in dog or human bone marrow or peripheral blood preparations. Correlation between the lupus erythematosus test and the clinical and laboratory aspects of 22 patients with acute or subacute lupus erythematosus was reported. A report is made of investigations into the identification of the lupus erythematosus factor.

*Discussion*

(Dr. Tom R. Hamilton, Minneapolis, Minn.) Was heparin necessary, or did you use citrated blood?

(Dr. C. Bruce Taylor, Chicago, Ill.) Does freezing of the serum affect the lupus erythematosus phenomenon?

(Dr. Averill A. Liebow, New Haven, Conn.) I should like to ask Dr. Haserick whether he has tried the buffy coat of centrifuged blood of normal persons as a test medium for the production of the "lupus erythematosus cell." Recently one of our students has successfully attempted this procedure using the plasma of 2 patients with disseminated lupus erythematosus. Has Dr. Haserick any data from his abundant material to confirm or deny these findings?

(Dr. Howard C. Hopps, Oklahoma City, Okla.) Has Dr. Haserick added relatively large amounts of normal gamma globulin to the cells to see whether the effect can be produced?

(Dr. Haserick) In reply to the question on the use of heparin and citrate as anticoagulants, I think heparin produces a much better preparation, though the other anticoagulants seem to work fairly well. Freezing of the serum has not affected the lupus erythematosus phenomenon.

In answer to Dr. Liebow's question, it is possible to use the buffy coat of peripheral blood and lupus erythematosus plasma, but in my experience the results have been inconsistent. The peripheral blood technic has been described by Moffat, Barnes and Weiss in whose hands it has been very effective. If positive, the result would be significant, but I have had false negative results in peripheral blood preparations, whereas marrow gave a positive result. At the Cleveland Clinic, dog marrow is so conveniently supplied that we continue to use it as an indicator. We have tried the normal gamma globulin, without any effect in inducing the lupus erythematosus phenomenon.

GLYCOPROTEINS IN HUMAN PATHOLOGIC THYROID GLANDS. G. Milles and (by invitation) I. Gersh, Chicago, Ill.

*Abstract.* Thyroid glands obtained by surgical excision were prepared by freezing and drying. Paraffin-embedded sections were stained by the Hotchkiss technic for glycoproteins before and after treatment designed to extract thyroglobulin. Findings were correlated with the clinical and microscopic diagnoses and preoperative therapy. Variations were noted in intracellular and alveolar glycoprotein under varying conditions. A particularly dense form of glycoprotein, which on the basis of extraction may be largely thyroglobulin, was identified in certain specimens.

*Discussion*

(Dr. Ralph D. Lillie, Bethesda, Md.) I presume that the glycoprotein material has had further identification by chemical tests, other than giving the $HIO_4$ Schiff reaction.

(Dr. Milles) No, the material has been extracted, and the extractable material has been studied from the standpoint of its resemblance to thyroglobulin. It has the same ultraviolet absorption curve and the same iso-electric point. There are several other details concerning this I could not go into because of lack of time; the work was done by Dr. Gersh and is in press now. Specifically, as to calling it glycoprotein, perhaps we are stretching it to use that term without quotations marks, as the only specific basis for calling it glycoprotein is its stainable quality.

(Dr. Lillie) We are becoming a little dubious of that.

(Dr. Milles) I realize that.

TISSUE MAST CELLS WITH ANITSCHKOW NUCLEI: INVESTIGATIONS INTO CYTOLOGIC BASES OF RHEUMATIC PROCESSES. **Tom R. Hamilton and (by invitation) Jerome T. Syverton, Minneapolis, Minn.**

*Abstract.* The rôle of the host in production of changes of the rheumatic type is being investigated by histochemical means. The object is to study the relationship of age and species to tissue susceptibility and to learn how tissue cells contribute to rheumatic processes. Tissues for study were provided by hearts from 12 patients dead of rheumatic fever, 19 patients as a control series, and 18 monkeys from 4 different colonies. Sections of heart were prepared from seven areas designated by Gross *et al.* as the most frequent sites of rheumatic activity. Especial attention was given to alterations in vascular and supporting elements by employing the alcoholic toluidine blue method of Sylven to demonstrate sulfur-containing polysaccharides by metachromasia and by utilization of the technics of Hotchkiss and McManus for polysaccharides in general.

Metachromasia was observed as a prominent feature at the juncture of valvular tissue and underlying cardiac structures; especially the left auricle; at the juncture of endocardium with myocardium; in perivascular areas of the myocardium; in vascular areas of the pericardium. The patterns of distribution of tissue or histogenous mast cells, Anitschkow cells, and metachromasia were essentially in agreement. A quantitative estimation of the number of tissue mast cells where they are most concentrated was made by enumerating these basophilic tissue cells in five adjacent high-power fields. Of especial interest were the rheumatic hearts from 9 children within the age group of the 19 controls. The median number of histogenous mast cells was 23 for the 9 with active rheumatic fever and 12 for 19 non-rheumatic controls. More extensive investigation is warranted by the "t test" which yielded a P value of 0.11.

An association of metachromatic cytoplasmic granules typical of mast cells with a nucleus of the Anitschkow type was observed in 53 cells in cardiac tissues of human and monkey origin. The occurrence in acute rheumatic fever of tissue mast cells with an Anitschkow type of nucleus was established by statistical analysis as highly significant; 8 of 9 such hearts showed this association in contrast to only 6 of 19 controls. The $\chi^2$ value was 8.023 and $P = 0.005$. Twenty of these cells in 9 hearts of the test group gave a mean of 2.2, while 14 in 19 control hearts gave a mean value of 0.73. Cells were observed which apparently are in a series from tissue mast cells with abundant cytoplasmic granules to those in which a loss of granules uncovers a linear bar of chromatin characterizing the Anitschkow nucleus. This evidence and the characteristic scanty or absent basophilic cytoplasm indicate the identification of some Anitschkow cells as a form of tissue or histogenous mast cells (basophilic tissue cells, "mucinoblasts," "heparinoblasts"). This suggests that de-

granulation of tissue mast cells with liberation of highly reactive acid mucopolysaccharide, such as heparin, may reflect participation by the host in the pathogenesis of the focal rheumatic process. This concept receives support from activities attributed to heparin, such as the inhibition of hyaluronidase and the antihistaminic effect, and by increased tolerance to heparin in acute rheumatic fever.

*Discussion*

(Dr. Jerome Gross, Cambridge, Mass.) In connection with the point on the appearance of metachromasia in pathologic lesions, I think it has been shown by Sylvia Bensley and others that in healing processes, that is, when fibrinogenesis occurs, we also have the laying down of metachromatic material. Probably in the pathologic lesion there is some healing going on, and I wonder if it is possible to separate the metachromasia as a consequence of the normal healing process from that in any pathologic process. Secondly, I believe Sylven and others pointed out that mast cells accumulate around tumors and some inflammatory processes as well. I wonder how specific is this accumulation of mast cells around nodules in rheumatic conditions.

(Dr. Joseph C. Ehrlich, New York, N.Y.) I should like to ask Dr. Hamilton if any digestion experiments with ribonuclease were carried out. I have followed some of the changes in these cells with the ultraviolet microscope, and also with histochemical technics, including the Feulgen reaction, methyl green-pyronin, and enzymatic digestions. The evidence indicates that as the cytoplasm of Anitschkow cells increases in amount, basophilic substances which accumulate absorb very strongly at 2537 Å. Pyronin staining of the cytoplasm is intense and is abolished after digestion by ribonuclease. The intranuclear body in these cells also absorbs intensely at 2537 Å and is Feulgen-positive. I have not been able to find pyronin-positive nucleoli in the nuclei of Anitschkow cells.

(Dr. Otto Saphir, Chicago, Ill.) In certain types of myocarditis I also have seen a very marked increase in mast cells, and I wonder whether this occurs only in certain myocarditides. Have you found the same, or have you observed this increase only in rheumatic processes?

(Dr. Hamilton) In reply to the first question, we were of the opinion at first that this numerical increase in tissue mast cells was associated more with the healing process or laying-down of tissue, and that in such a long insidious process as rheumatic fever the increase was reflected also in the free metachromasia, but there was no proved specific lesion or phenomenon of specific pathologic nature in the simple increase in typical mast cells *per se*.

In regard to the occurrence of mast cells around tumors, we have felt that the occurrence probably has a relationship with new growth, and we are familiar with that association, not only as cited by Sylven, but by other observers who have described mast cells as frequently occurring around tumors.

With reference to the treatment with ribonuclease, I have not tested this cardiac tissue with that enzyme.

In regard to Dr. Saphir's question concerning mast cells in myocarditides, we have seen mast cells in animals as well as in the human, and we have studied the mast cells in myocarditis in monkeys. That is not the subject of this paper, but there were about 18 per 5 high-power fields by our counts; however, as yet I have not broken down all of those findings.

LESIONS OF THE HEART IN RABBITS INTRAVENOUSLY INJECTED WITH SERUMS FROM CASES OF RHEUMATIC FEVER. Mark P. Schultz and George L. Fite, Bethesda, Md.

*Abstract.* Experimentally produced myocardial and vascular lesions observed in hypersensitive animals have been the subject of acute interest since the publications of Rich and Gregory in 1943. Some have resembled the lesions of periarteritis nodosa, others the lesions of rheumatic fever. In the present investigations rabbits

were given intravenous injections of acacia, followed by multiple injections of rheumatic fever (human) serums at intervals of a few days to a week, the rabbits being sacrificed about 3 months later after 7 to 12 serum injections. In a substantial number of animals lesions were observed in branches of coronary arteries of varying size, which correspond to rheumatic arterial lesions described in the human being. Endocardial changes were rare and Aschoff bodies were not observed. Animals receiving normal human serum showed only a trace of such changes. It is suggested that the use of rheumatic fever serums as sensitizing agents yields an interesting corollary to investigations along these lines in which other sensitizing agents were used.

ELECTRON MICROSCOPIC STUDIES OF COLLAGEN FROM NORMAL AND DISEASED TISSUES. James C. Gale (by invitation), Detroit, Mich.

*Abstract.* Electron microscopic studies of collagen fibrils and filaments have been made from normal and diseased human tissues, including certain of the "collagen diseases," and from animal tissues. An effort has been made to elucidate further the ultra-structure of the collagen fibril and filament and to make a comparative study of fibrils from normal and diseased tissues. Present electron microscopic technics limit the comprehensiveness of such a comparative study, but permit certain tentative conclusions. Collagen fibrils were isolated from tissues involved in acute rheumatic fever, scar tissue from old rheumatic fever, rheumatoid arthritis, lupus erythematosus, keloid, cirrhosis of the liver, fibroma, neurofibroma, fibrous adhesion of lung, and fibrosarcoma. Fibrils so far isolated revealed no marked change in structure from those of normal tissues. The interfibrillar ground substance varied considerably as to the ease and completeness with which its interference was eliminated and proved the chief obstacle to a more satisfactory comparative study of fibrils from the tissues studied.

*Discussion*

(Dr. Samuel K. Elster, Washington, D.C.) It must be pointed out that when similar patterns from collagen fibrils in various conditions are obtained, it should be considered that small areas of abnormal collagen may be missed. Until the ultramicrotome is developed to such a point that we can select areas for histologic section and then transpose them to the electron microscope and see what the collagen looks like, it is not fair to say that all collagen from pathologic material has a periodicity of 640 Å units. I have had occasion to examine a subcutaneous nodule from a person who had rheumatoid arthritis, and histologic examination revealed a typical fibrinoid necrosis of the collagen. Using a fragmentation technic and trying to sample randomly the collagen fibrils in the electron microscope, I found some collagen fibrils that did not appear normal. For the most part, the fibers were broad and had the typical periodic structure of 640 Å units. Some fibrils were much thinner and displayed a prominent intraperiod structure of 3 bands. In places, this intraperiod structure obscured the over-all periodicity, so that it appeared as though the periodicity of these fibers was about one-third of 640 Å or 210 Å units. This has been reported as the periodicity for fibrin. It is not intended to imply that these fibrils are fibrin, but merely to point out that the typical electron micrographs of collagen were not obtained.

(Dr. Gale) There are many drawbacks to a comparative study of collagen fibrils with present electron microscopic technics. I regret that I did not have time to go into some of the difficulties involved. I meant only to point out that in so far as could be determined from our work no actual structural differences could be found in the fibrils from normal and diseased tissues. I meant also to indicate that before any real conclusions can be drawn numerous studies must be made. As many as five rings can be seen within some of the periods of normal collagen fibrils in the second lantern slide shown.

A Study of the Aging of Collagenous Connective Tissue of Rat Skin with the Electron Microscope. Jerome Gross (by invitation), Cambridge, Mass.

*Abstract.* X-ray diffraction and electron microscope studies have established a relatively well defined morphologic pattern for collagen, namely a characteristic wide angle x-ray pattern and an axial long spacing in the fibrils averaging 640 Å. Histologic silver staining methods have established the overwhelming predominance of argyrophilic "reticulin" in the newborn rat skin, with gradual transition to typical collagen with aging. Electron microscope examination of the newborn rat corium shows the presence of typical non-branched fibrils with average axial period of 640 Å and intraperiod structure characteristic of mature collagen. These fibrils differ from the latter in width, ranging in gaussian distribution from 300 to 700 Å with a peak at 500 Å. Adult rat skin collagen ranges from 800 to 1800 Å, averaging 1300 Å. There is a variation in these figures of about 200 Å units for different animals of the same age group. The width distribution curve for fibrils from each of the skins of rats ranging in age from 18 to 60 days is spread irregularly from 400 to 2000 Å with the broader fibrils predominating with advancing age. Preliminary x-ray diffraction studies of the newborn rat skin give typical wide angle collagen patterns. Investigation of silver staining indicates that the fibrils adsorb metal colloids. Some properties of the ground substance are discussed.

*Discussion*

(Dr. Peter Gruenwald, Brooklyn, N.Y.) I wonder if it would be desirable or feasible to lay down some criteria for collagenous connective tissue, since different ordinary staining methods will stain varying numbers of fibers in the same tissue.

(Dr. Samuel K. Elster, Washington, D.C.) Did you find any relationship between the width of the fibril and the length of treatment with trypsin prior to use in the electron microscope?

(Dr. Gross) In reply to Dr. Gruenwald's question, connective tissue stains stain only bundles of collagen fibrils which include substances other than collagen, so that staining alterations do not necessarily indicate changes of the protein collagen. For this reason more distinctive fingerprints for the connective tissue components are essential.

Dr. Elster asked about the effect of trypsin. It does not seem to make much difference how long you trypsinize a particular preparation. The collagen fibrils seem to be about the same and uniform in width. Trypsinization does not seem to affect the structure of the collagen fibril. Although there is some evidence that it may affect the intermolecular bonding of the fibril, it is not evident in this type of preparation.

Adrenal Cortical Activity in Relation to Lymphoid Tissue and to Immune Bodies. H. C. Stoerk and (by invitation) M. Solotorovsky, Rahway, N.J.

*Abstract.* Alterations in lymphoid tissue produced by defective and excessive adrenal cortical activity were studied in partially starved rats. Changes in thymic weight were roughly paralleled by changes in other lymphoid organs. The weight and histologic features of thymus, lymph node, and spleen were compared in the various experimental groups. The changes observed were more pronounced and more highly reproducible in thymic than in other lymphoid tissue. No loss of thymic tissue occurred in adrenalectomized, in adrenalectomized diabetic, or in adrenalectomized and thyroidectomized rats on restricted food intake for 5 days. In sham operated rats under the same conditions a reduction in thymus weight of about 30 per cent occurred. In adrenalectomized rats, 5 daily injections of 2 mg. of cortisone caused a reduction in thymic weight of about 60 per cent. Since in these atrophic glands mitotic activity was undiminished, the loss of thymic tissue appeared predominantly due to an increased destruction of lymphocytes.

Suspensions of lymphocytes, obtained from rat thymus, when injected into rabbits gave rise to antibodies which strongly agglutinated rat lymphocytes but failed to react with rat gamma-globulin. Conversely, a potent rabbit antiserum against rat gamma-globulin failed to agglutinate rat lymphocytes and gave no precipitins with extracts from thymic lymphocytes. Immune responses including the "anamnestic response" were unimpaired in adrenalectomized rats and mice. Therefore the inability of adrenalectomized animals to break down lymphocytes was not associated with impairment of immune responses. In previously immunized rabbits a single injection of cortisone did not produce an "anamnestic rise" but actually was followed by a drop in circulating antibody as determined by quantitative measurements.

THE EFFECT OF CORTISONE ON THE LESIONS OF PERIARTERITIS NODOSA. Archie H. Baggenstoss and (by invitation) Richard M. Shick and Howard F. Polley, Rochester, Minn.

*Abstract.* This report is based on 2 cases of periarteritis nodosa in which the patients were treated with cortisone acetate. The clinical diagnoses were confirmed histologically by biopsy. Both patients were critically ill when treatment was begun. Dosage schedules varied, depending on the clinical and biochemical response and the results of subsequent biopsies. Both patients experienced prompt subjective relief of symptoms after administration of the hormone. Fever subsided within 24 to 72 hours, and the sedimentation rate of the erythrocytes decreased to normal more gradually. Partial relapse occurred after administration of the hormones was discontinued and improvement recurred after administration of the hormone was resumed. In the first case, the patient was observed for 75 days and received 3.62 gm. of cortisone; in the second case, the patient was observed for 146 days and received 13.35 gm. of the hormone. Despite initial improvement, both patients died of cardiac and renal failure.

In both cases, necropsy disclosed numerous old and recent infarcts which were scattered throughout many organs but were particularly numerous in the heart, kidneys, and gastro-intestinal tract. The patient who received the more cortisone had gross atrophy of the suprarenal glands. Histologically, the most remarkable finding was the apparently complete healing of all the arterial lesions. All histologic signs of inflammation had disappeared in the first case within 3 weeks, and in the second case within 3 months, after biopsy had disclosed acute arteritis. Histologic examination of multiple sections from all organs failed to reveal a single vessel which was the site of active inflammation. However, fibrous obliteration of vessels occurred and resulted in infarcts, which were particularly numerous in the kidneys, heart, and intestinal tract, but also were present in the liver and suprarenal glands. The healed arterial lesions were characterized by severe intimal fibrosis, by focal defects and sometimes complete destruction of the elastic lamellae, and by fibrosis of the media, adventitia, and perivascular tissue in which phagocytes containing hemosiderin were observed. The intimal fibrosis resulted in practically complete occlusion of the lumen in many arteries. In the second case, the histologic findings differed from those in the first case in that aneurysm formation and thrombosis were more common. In both cases, histologic examination of the suprarenal cortex revealed a moderate degree of atrophy and a decrease in lipid content.

THE EFFECT OF THE HYPERADRENAL STATE ON CONNECTIVE TISSUE. Charles M. Plotz (by invitation), Edward L. Howes (by invitation), Karl Meyer, James W. Blunt (by invitation), Raffaelo Lattes and (by invitation) Charles Ragan, New York, N.Y.

*Abstract.* Cortisone and pituitary adrenocorticotrophic hormone (ACTH) have been shown to act favorably on diseases of mesenchymal tissues. During the course

of treatment of patients with these hormones, marked delay in healing of spontaneous and incised wounds was noted. This led to an experimental evaluation of the effect of the steroid hormones on experimental wound healing. Several technics were employed, including skin defects, fractures, and incised wounds of skin and stomach. In all, the administration of cortisone resulted in a delay of appearance of new forms of connective tissue compared with control animals. Other steroid hormones tested had no such effect. It is suggested that the mode of action of cortisone and ACTH on the "mesenchymal diseases" is related to this depressive effect on connective tissue. This results in a decrease of host reactivity and disappearance of symptoms and signs related to it during the administration of the hormones.

*Discussion*

(Dr. Paul R. Cannon, Chicago, Ill.) One of the disturbing features of these studies is the evidence that cortisone and ACTH may bring about a negative nitrogen balance, with an increased urinary excretion of amino acids, and interference with tissue protein synthesis. Do you suppose that, in many of these studies, excessive doses are being used and that this may explain in part the inhibitory effects?

(Dr. B. Black-Schaffer, Durham, N.C.) I wonder if you will comment on the possible relationship between your work and vitamin C deficiency as produced by Wolbach. Some of your pictures remind me of the experiments in scurvy.

(Dr. Plotz) In answer to Dr. Cannon's question about the negative nitrogen balance, we have not done balance studies on our animals. We have produced negative nitrogen balance in other ways, and we have not been able to reproduce this effect on wound healing. We tried to affect the negative nitrogen balance to some extent by administering testosterone along with cortisone and have been unable to counteract the influence on wound healing.

As far as dosage is concerned in the human, there seems to be a fairly critical level below which we will not get any beneficial clinical effect and we have felt it was necessary to make the human organism hyperadrenal. We have made the animals hyperadrenal, giving them doses even greater than the comparable human dose. It is of interest, however, that we were able to get to a dose fairly close to the comparable human dose and still were able to produce an inhibitory effect on healing, even though it was not as dramatic.

In reply to Dr. Black-Schaffer, we have been interested in the vitamin C question because the effect on healing of scurvy has been so similar to that produced by us. I can only tell you what we have observed in the matter. In the first place, we have done vitamin C levels on some of our animals and as far as we could tell they were not scorbutic. Also we have tried to flood human beings who were getting cortisone or ACTH with vitamin C, to see if we could block the effect of the hormones in that manner, and we have been unable to do so.

THE EFFECT OF CORTISONE ON THE FORMATION OF GRANULATION TISSUE IN MICE. David M. Spain and (by invitation) Norman Molomut and Alvin Haber, Valhalla, N.Y., and Brooklyn, N.Y.

*Abstract.* The effects of cortisone on the alleviation of the clinical manifestations of the collagen diseases have led us to an investigation of some of its biologic effects on granulation tissue, the reticulo-endothelial system, and on the formation and fate of acute inflammatory exudate. This study is primarily concerned with the effect of cortisone on the formation of granulation tissue. Ragan and his associates observed a retardation of wound healing in patients under treatment with ACTH. They also observed a depression in the formation of granulation tissue in 4 rabbits under the influence of cortisone. They did not observe this effect in rats.

In this study 40 white albino mice (20 to 25 gm.) housed in individual cages and kept under controlled conditions were wounded on their backs so that each mouse had two identical round wounds 0.5 cm. in diameter. Twenty mice were injected

subcutaneously with 1 mg. of cortisone twice daily (injections beginning one day previous to wounding). The 20 control mice were injected with the diluent twice daily. Four animals in each group were sacrificed at 24-hour intervals following wounding. The wounds were completely excised, sectioned, and stained with hematoxylin-eosin and toluidine blue. Complete autopsy was performed also on each animal. Microscopic examination of these wounds revealed a complete suppression of all elements in wound healing of the cortisone-treated group as compared with the control group. Wounds examined after 24 hours (8 wounds in each group) revealed an almost complete lack of exudate and fibrin in the cortisone-treated group. Cellular elements were markedly diminished. As the days progressed, comparable studies revealed practically no new capillary formation, sparse fibroblastic proliferation, and insignificant ground substance present in the cortisone-treated group. By the fifth and final day of this study, the majority of the wounds in the control group showed considerable compact, well vascularized granulation tissue—almost, if not completely, covered with epithelium, whereas in the cortisone-treated group scant collections of fibroblasts were present. However, epithelization in some wounds was complete, having practically grown over a bare adipose tissue surface. The effect on the healing of wounds was in many respects similar to that seen in vitamin C depletion, in particular, the absence of ground substance as demonstrated by the toluidine blue stain. An interesting side observation was that the spleens of the cortisone group were one-fifth the size of the control group.

In another group of mice the same dose of cortisone was administered 48 hours after wounding. Treatment was continued for 5 days. Comparison with controls in this group revealed no significant difference in the quantity or quality of the granulation tissue. Administration of 10 mg. daily of ascorbic acid in animals pretreated with cortisone did not effect the inhibition of granulation tissue formation.

### *Discussion*

(Dr. Charles M. Plotz, New York, N.Y.) I would like to ask if epithelization was found in all of the mice, as was shown in some. The reason for that question is that we are hearing of some patients on cortisone or ACTH who have wounds and are said to show prompt healing. We found that the skin heals promptly but when the wound is biopsied, there is very little healing in the depths of the wound.

(Dr. Spain) The epithelization that was present in the animals pretreated with cortisone was not as marked as in those with considerable granulation tissue, nor was it as cellular.

### Effect of Ascorbic Acid Deficiency on the Collagen Content of Guinea-Pig Tissues. Samuel K. Elster (by invitation), Washington, D.C.

*Abstract.* Ascorbic acid has been demonstrated to be essential for the formation of connective tissue in the guinea-pig. Its rôle in the maintenance of pre-formed connective tissue has not been well elucidated. Male guinea-pigs weighing approximately 200 gm. were placed on a vitamin C-deficient diet supplemented with the other known accessory growth factors. These animals developed signs of scurvy in 3 to 5 weeks at which time they were sacrificed. Chemical measurements of the collagen content of the lungs, liver, kidneys, spleen, heart, and adductor muscles of the leg were made. Similar determinations were performed in groups of age and weight controls. The following results were obtained:

1. There was significantly less collagen in the lungs, liver, and kidneys of the experimental group than in similar organs of the age control group. However, these values were not significantly different from those of normal animals of the same weight.

2. The collagen content of the spleen was not altered.

3. There was a relative (percentage) increase of collagen in the heart and muscle of the scorbutic animals over that of either control group. These observations are

taken to indicate that there is not selective loss of collagen in the scorbutic state and that ascorbic acid may not be essential for the maintenance of connective tissue.

*Discussion*

(Dr. John W. Harman, Madison, Wis.) The methods of Lowry used here were tried in our department. We found it necessary for micro-procedures to adopt nitrogen estimations rather than a weight basis. I would like to know whether that was necessary in your particular investigation. Further, in our studies on the normal human heart with aging, we observed it was probably more important in considering collagenous aging to take it as a proportion of the total protein on a dried weight basis rather than on the total weight of the particular organ, and we have an index which we called the collagen index. Our studies showed that in the auricles of the heart there was no change from birth to 80 years of age, but in the ventricles there was a gradual collagenization. We have no guarantee, of course, except for the normal condition grossly of these hearts that they were all normal in vitamin intake, but this might have some bearing on your particular findings, whether or not, quite apart from a lack of anabolism, there may be a decrease in catabolism in aging in the ventricles as compared with the atria.

(Dr. Elster) In reply to Dr. Harman's question, we found after several months of trial that by suitable alteration of Lowry's technic we were able by this direct quantitative method to use weight bases and obtain fairly reproducible results without resorting to Kjeldahl determinations. We did not do nitrogen determinations as controls. One of the things that disturbed me during the course of the experiment was whether in calculating the result in terms of collagen per wet weight we were getting the total amount of collagen in the body. I have not presented the figures, but I did calculate on another group of scorbutic animals the amount of water in the tissues and found it no different from normal animals for the same weight and age. I did not do the water content and collagen content on the tissues in the same experimental animals because I wanted to use the entire organ, and I wanted to see whether relative or absolute figures would be more valid. I feel that one can draw conclusions better from absolute than from relative figures.

THE CONCEPT OF COLLAGEN DISEASE. Paul Klemperer,* New York, N.Y. This paper appears in this issue of *The American Journal of Pathology* (page 505).

THE PROBLEM OF DEPLETION OF THE PROTEIN RESERVES. Paul R. Cannon, Chicago, Ill.

*Abstract.* The problem of the metabolic rôle of the protein reserves has aroused much interest since Whipple and his associates directed attention to the pathologic consequences of their depletion. These reserves constitute a potential source of building materials and enzyme systems for metabolic purposes. The mammal exhibits a remarkable capacity to resist depletion of these reserves and it requires considerable periods of time to accomplish this. In general, the degree of hypoproteinemia indicates the degree of depletion of the protein reserves. In moderate degrees of hypoproteinemia, however, the usual processes of anabolism may be retained to a remarkable extent; but below a total protein concentration of 5 gm. per 100 cc. of plasma, and particularly below 4 gm., the evidences of approaching exhaustion of the protein reserves become more apparent. It is here that pathologic manifestations may become evident. It is probable that, as in the case of vitamin deficiencies, where pathologic manifestations do not become prominent until the bodily stores have been severely depleted, so with the protein reserves not much abnormality of structure or function is to be expected with the milder degrees of protein depletion. Discussion is directed to the factor of time in the induction of severe depletion of the protein reserves and to some of the consequences of this depletion.

*By special invitation of the Council.

*Discussion*

(Dr. Shields Warren, Boston, Mass.) I should like to ask in the matter of antigen production whether it is an over-all phenomenon, or whether there is a variation between the antigenic response in one or another organ.

(Dr. Paul Klemperer, New York, N.Y.) I think I understood Dr. Cannon to make reference to enzyme studies, and I should like to hear more about that.

(Dr. Norbert Enzer, Milwaukee, Wis.) Is it possible that in the testicle, particularly, loss of basement membrane and supporting stroma might account for the widening of the space between the tubules and thus give the effect of parenchymal atrophy?

(Dr. Cannon) In answer to Dr. Warren's question, there is reason to believe that following intravenous immunization most of the antibody comes from the spleen. This problem is discussed by Rowley in the April issue, 1950, of the Journal of Immunology. Undoubtedly there is also release of antibody from the liver and the reticulo-endothelial system in general. We assume, however, that when we are trying to measure a general effect it is probably better to use the intravenous method. In our studies the most striking fact is the length of time required on a diet devoid of protein to cause a marked depletion of the protein stores. In this country we do not see many patients so severely depleted, and when those with total protein concentrations below 5 gm. per 100 cc. of plasma are encountered, we do not consider it humane to continue them on low protein diets as experimental subjects.

In regard to Dr. Klemperer's question, I would say that at least a dozen enzyme systems have been studied and shown to be reduced in severe protein depletion.

In regard to the effect on the testicle, I believe that most of it is parenchymal.

In view of the current emphasis on calories, I would like to emphasize the fact that in these experiments the diets were isocaloric, so that the effect is mainly that of protein depletion.

HEPATIC NECROSIS INDUCED BY DIETARY MEANS. M. R. Abell (by invitation) and J. M. R. Beveridge (by invitation), London, Ont.

*Abstract.* One hundred and twenty-one male rats were fed a diet low in protein (7.2 per cent yeast protein) and deficient in alpha-tocopherol. This diet has been shown to produce necrosis in 19 of 20 animals in an experiment terminated at 105 days. Small groups of rats, selected at random, were sacrificed at intervals throughout an experimental period of 92 days. The cytologic changes occurring prior to and at the time of development of necrosis were studied by means of sections stained with hematoxylin and eosin, Masson's trichrome, Best's carmine, and scharlach R stains. The composition of the livers with respect to the following components was determined: moisture, glycogen, total lipids, total fatty acids, neutral fat, phospholipids, total cholesterol, free cholesterol, and cholesteryl esters. During the pre-necrotic period, the hepatic cells showed a prominent loss of cytoplasm and a decrease in basophilic granules attributable to a loss of protein. There was a progressive increase in stainable lipid material in the liver cells about the central veins. The livers which presented evidence of necrosis were divided on the basis of gross and microscopic findings into those showing massive necrosis, sub-massive and recurrent sub-massive necrosis, and post-necrotic scarring.

Biochemical investigations revealed a series of interesting changes. During the pre-necrotic period there was an initial and proportional decrease in both dry and wet liver weight. The glycogen values remained relatively constant. There was a progressive rise in total liver lipids, neutral fat, and cholesteryl esters. Phospholipid and free cholesterol values remained constant, thus indicating that the change in total liver lipids was due to an increase in neutral fat and cholesteryl esters. The necrotic livers differed chemically from the non-necrotic by showing a marked increase in water content, a striking increase in free cholesterol, and an equally pro-

nounced decrease in phospholipid and glycogen levels. There was no additional change in total lipid, nuetral fat, and cholesteryl ester values at the time of necrosis.

*Discussion*

(Dr. Paul R. Cannon, Chicago, Ill.) I would like to ask Dr. Abell if he can explain his success in getting massive necrosis of the liver when so many other workers have failed. Several workers, including Dr. Jaffé in our laboratory, have failed to secure the massive dietary necrosis of the liver in rats. Is it due to the absence of tocopherol, or to a difference between English and American yeasts?

(Dr. Hans Popper, Chicago, Ill.) I would like to ask the same question that Dr. Cannon did because we also know of several futile attempts to produce hepatic necrosis with American yeast whereas administration of British yeast was successful. I believe that the marked fatty changes shown here have usually not been seen in animals in which necrosis developed after yeast administration. Was ceroid found in the livers of animals used in this experiment? On the usual lipotropic diets, ceroid is rather commonly found, whereas, it is not seen in massive hepatic necrosis. I finally would like to ask whether the lesion presented can be prevented by administration of tocopherol.

(Dr. Conrad L. Pirani, Chicago, Ill.) Were the incidence and severity of these lesions greater in the left lobe as compared to the right lobe of the liver?

(Dr. Robert E. Stowell, Kansas City, Kans.) I would like to know if these animals were fasted to eliminate variables due to dietary factors before they were killed for the chemical study. I should also like to inquire whether the general health of the animals with and without hemorrhage in the liver was fairly comparable, since differences in health could produce some chemical changes in the liver.

(Dr. G. J. Dammin, St. Louis, Mo.) I would like to know whether dye-excretion studies were made while the animals were on the diet. I have in mind the paradoxical finding in fatty liver that there may be a more rapid removal of dye than normal, somewhat contradicting the concept that there is an impaired circulation in the sinusoids of the fatty liver.

(Dr. Peter P. Ladewig, Montgomery, West Va.) In experiments carried out some years ago on fasting guinea-pigs we found a peculiar discrepancy between the decrease of the total liver volume as well as the total cytoplasmic mass of the liver and a marked increase in the total volume of the liver cell nuclei. This increase was evidenced by an absolute numerical increase in polygonal cells of greatly reduced cytoplasmic volume, an increase in double- and multi-nucleated cells, and a marked increase of the means of the volume of the liver cell nuclei. The increase in the absolute volume of nuclear material started after a period of latency of between 12 to 18 hours, and attained its maximum about 48 to 72 hours after the onset of fasting, reaching up to 180 per cent of the original total and absolute nuclear volume. At this time the cytoplasmic volume had dropped to an average of 36 per cent of its original volume. From then on, up to shortly before death of the animal, it remained stable only to show a steep drop within a few hours preceding death.

(Dr. Abell) We have used Fleischmann's active baker's yeast in most of our experiments but have obtained the same incidence of necrosis with inactivated yeast. With brewer's yeast a high incidence of necrosis was obtained, but after a longer feeding interval. We have also produced massive necrosis by feeding diets in which 4 per cent casein was the sole source of protein. The incidence of the lesions was less than that obtained with the yeast diets.

The age of the animals is an important factor. In weanling male rats, necrosis appeared at an average of 36 days; in young male adults, averaging 129 gm. in weight, necrosis appeared at an average of 58 days; whereas in adult rats, average weight 235 gm., 18 of 20 animals died of necrosis after an average of 205 days on the test diet.

The inclusion of cod liver oil in the diet as a source of vitamins A and D increases the incidence of necrosis. In animals that received crystalline vitamins A and D the incidence of necrosis was approximately half that of a group which were fed a similar diet containing 2 per cent cod liver oil.

We have previously pointed out that the failure of some investigators to induce necrosis was due to the inclusion of alpha-tocopherol or a fat of high tocopherol content in their diets. In addition, there may be other factors involved, such as vitamin $B_{12}$ or folic acid, a possibility which we are at present investigating.

In answer to Dr. Popper's question, we found no evidence of ceroid in livers showing massive necrosis. However, in livers which showed recurrent submassive necrosis and post-necrotic scarring, there were masses of ceroid associated with the areas of fibrosis. There was no evidence of ceroid in the pre-necrotic livers. Supplements of choline chloride reduced the fatty infiltration during the pre-necrotic period, but did not affect the development of necrosis. The addition of 2 mg. of alpha-tocopherol daily by mouth completely prevented the development of necrosis.

In reply to Dr. Pirani, the necrosis in livers displaying evidence of a single episode of submassive involvement was limited to the left half of the liver, the left lobe and the left half of the median lobe. Also, in the majority of livers showing recurrent submassive necrosis and post-necrotic scarring, the left half of the liver showed the greater degree of involvement. Himsworth and Glynn explained the predilection of the left half of the liver to initial damage on the basis of striation of blood flow within the portal system, a phenomenon described by Serege in 1901 and confirmed by Copher and Dick. They postulated, that as a result of this mechanism, protective substances absorbed from the small intestine were carried to the right half of the liver, thus conferring a greater degree of protection on this half of the liver.

In answer to Dr. Stowell, all animals selected for sacrifice throughout the experiment were fasted for a period of 10 hours. During the first 2 weeks of the experiment there was an average loss of 20 gm. of body weight following which little change was observed. The general health of the animals remained good until they developed necrosis. They ate their full allotment of diet and appeared healthy and active until within 12 hours to 3 days prior to death.

In reply to Dr. Dammin's question, no dye excretion studies were carried out in any of the animals in this experiment.

Dr. Ladewig, we did not do nuclei counts, but our impression was that during the pre-necrotic period, the cytoplasm of the liver cells was decreased in amount, the nuclei being more closely packed than in the normal livers. The cells resembled those termed "hypocytoplasmic" by Kosterlitz.

LIVER DAMAGE IN ULCERATIVE COLITIS. Paul Kimmelstiel and (by invitation) H. Lee Large, Jr., and Hugh D. Verner, Charlotte, N.C.

*Abstract.* The scant literature concerning the relationship between ulcerative colitis and liver damage reveals wide diversity of opinion. We are presenting a study of 93 livers of patients with non-specific ulcerative colitis, 81 of which are from the files of the Armed Forces Institute of Pathology and 11 from Charlotte Memorial Hospital. We observed a variety of significant lesions in 40 per cent of all cases. Thirty-nine per cent of the lesions were classified as degenerative, 15 per cent as inflammatory. Some of the cases showed more than one type. The degenerative type of liver damage included frank cirrhosis, marked pseudo-lobulation without fibrosis, multiple bile casts, necrosis, dissociation, and fatty changes. Chronic degenerative changes such as cirrhosis and pseudo-lobulation were only rarely observed; severe fatty changes (in contrast to previous reports) were found in only 15 per cent, and acute regressive changes in approximately 8 per cent of the cases. Inflammatory lesions included chronic, severe, focally and diffusely distributed, interlobular hepatitis which was observed in 10 per cent of the cases. This lesion, not biliary in origin

and only in later phases associated with metallaxis of liver tissue, is apparently portal and probably embolic in origin.

Although the clinical charts were incomplete, definite evidence of functional impairment was noted in 10 per cent of the cases. Correlation of the morphologic changes and clinical signs and symptoms indicates that the degenerative liver changes are related to recurring attacks of diarrhea and are probably metabolic in origin. It is assumed that most of these changes are transitory in nature.

The liver changes in ulcerative colitis constitute a heterogenous group of lesions. Although the pathogenesis is not uniform, the frequency of occurrence is not compatible with the assumption of mere coincidence. Their recognition is obviously important in regard to the medical and surgical management of ulcerative colitis.

*Discussion*

(Dr. Howard C. Hopps, Oklahoma City, Okla.) Was the fatty change in the liver associated with emaciation in these individuals?

(Dr. E. Stark, Burlington, Vt.) I should like to ask, in view of the fact that similar changes in the liver occasionally occur in routine autopsy material, if any attempt has been made to see these changes in a control series of non-ulcerative colitis cases.

(Dr. Shields Warren, Boston, Mass.) I should like to ask if, in the interlobular hepatitis, bacterial stains were carried out on the sections.

(Dr. D. Murray Angevine, Madison, Wis.) How many of these patients had abdominal surgery performed prior to autopsy?

(Dr. S. C. Sommers, Detroit, Mich.) I wonder if the authors will comment on the etiology and pathogenesis that they believe produced these liver lesions.

(Dr. Large) I should have pointed out that the clinical information is rather inadequate. We have not directly correlated the degree of emaciation with the degree of fatty change.

Dr. Stark, the findings we have presented are far out of proportion to what we have ordinarily encountered in our autopsy material, with the possible exception of liver cirrhosis. I do not know what the incidence of cirrhosis would be in our own over-all material, but the other changes are enormously greater in number.

Dr. Warren, we have not done bacterial stains; admittedly, we should. These patients presented no evidence of septicemia. We have many with negative blood cultures and at autopsy there was no finding to indicate septicemia. We have not, of course, proved or disproved portal septicemia.

Dr. Sommers, the etiology and pathogenesis of these lesions are a little bit beyond the scope of one morning. We have indicated, I think, that we feel some of these, like the fatty changes, may be on a nutritional basis. Concerning the foci of necrosis, we decline to make a statement at the present time. We have indicated what we think may be the etiology of the so-called interlobular hepatitis, namely, that it may be portal-embolic in origin. However, since portal phlebitis has been described in primary biliary affections, ours can be taken only as a possible explanation.

Dr. Angevine, as I recall it, approximately 8 or 9 of these cases had abdominal operations, usually of the bowel.

CORRELATION BETWEEN BIOPSY FINDINGS IN LIVER CIRRHOSIS AND CLINICAL AND LABORATORY FEATURES. Hans Popper, Sheldon S. Waldstein (by invitation) and Paul B. Szanto, Chicago, Ill.

*Abstract.* Various histologic features in 97 liver biopsy specimens of patients suffering from different stages of cirrhosis were analyzed and correlated with the clinical and laboratory findings, using statistical analysis. This was done in order to determine the morphologic features which coincided with greatest regularity with activity of the cirrhotic process; furthermore, to investigate the histologic features characteristic of particular types of cirrhosis, to choose the laboratory test

which indicates best the morphologic features of activity of cirrhosis and, finally, to evaluate liver biopsy in the recognition of this activity. Of the histopathologic features chosen, damage of the epithelial cells showed the best correlation with clinical or biochemical activity. Other features, like leukocytic infiltration, focal necrosis and increased cellularity of the portal triads, showed less correlation. If present, it was explained chiefly by the concomitant presence of liver cell damage. Features related to scarring and fibroplasia as well as fatty metamorphosis showed no correlation with clinical or biochemical activity. These observations implicate liver cell damage as the most important factor in determining the clinical activity of cirrhosis. Increased cephalin flocculation and thymol turbidity as well as serum albumin reduction and, to a lesser degree, serum globulin elevation, of a series of hepatic tests investigated, best indicate the presence of liver cell damage in cirrhosis and may thus serve as criteria for activity of the cirrhotic process. Presence of ascites showed good correlation with scarring and inflammation of the portal triads, fibroblastic activity, marked reconstruction, bile duct proliferation, and angiogenesis, but not with liver cell damage. The patients with established alcoholic history revealed, as a rule, lesser incidence of liver cell damage and higher incidence of diffuse fatty changes than those without. Jaundice correlated well with liver cell damage, but the histologic picture in cirrhosis with jaundice did not show anything characteristic except for the presence of bile pigment. The reason for the presence of marked jaundice or for the intrahepatic interruption of the biliary flow in cirrhosis (cholestasis) was not apparent from the morphologic analysis.

### *Discussion*

(Dr. Alfred Plaut, Topeka, Kans.) It has been my custom for many years to pay little attention to the so-called small round cell infiltration in the portal triads in people over 40. I would like to hear Dr. Popper's opinion whether we can maintain that, or whether we should assume that all these people must have had, at the time of death or at some previous time, a severe damage of their liver. I would further like to ask whether Dr. Popper has paid attention to the presence of glycogen nuclei, and I should like to hear about the eosinophil cells in the infiltrate in the portal triad.

(Dr. P. O'B. Montgomery, Dallas, Texas) Was any consideration given to the depth of the biopsy in relation to the capsule of the liver?

(Dr. G. J. Dammin, St. Louis, Mo.) In the March, 1950, issue of The American Journal of Medicine, Post and Rose compared the clinical activity of the disease with liver function tests and liver biopsy. They concluded that liver function tests were a better index of the clinical state than was the histologic lesion as observed in needle biopsy of the liver.

(Dr. Popper) In answer to Dr. Plaut, the question of portal inflammatory infiltration has concerned us for many years. It is important in the interpretation of liver biopsy specimens in which it is commonly found and cannot necessarily be associated with the present disease of the patient. In over 40 per cent of over 100 livers of young persons who died instantaneously (within 10 minutes following a crash) cellular infiltration was noted in some of the portal triads. Since most of the specimens were derived from soldiers dying in flying accidents, this strongly suggests that they were in good physical health and even respiratory infections could be excluded. However, in a few such instances extensive portal cellular infiltration was noted in almost every portal triad. I do not know the pathogenesis of these infiltrations but I do not feel that even leukocytic infiltration in the portal triads necessarily means that the person is sick. It obviously means something, but I do not think it has clinical significance. We have not noted glycogen nuclei in our biopsy specimens of cirrhosis even if McManus stains were used. We saw them in our autopsy cases although they were not extremely common. We observed eosinophils

in considerable amount intermixed with neutrophilic cells in about 10 per cent of the cases.

In reply to the question of Dr. Montgomery as to the depth from which the biopsy specimen was obtained and as to the dark cells below the capsule, I want to stress that we do not like to examine subcapsular biopsy material (as it is usually obtained by surgical excision), especially in cirrhosis, because of the marked distortion of the lobular pattern and the connective tissue proliferation normally occurring in this region. All the material reported here was obtained by needle, either by the Turkel or the Vim-Silverman needle, from parenchyma about 2 cm. below the capsule. I am not aware of special changes of the cytoplasm of the subcapsular cells, but in the individual lobules the cells bordering the portal triads, in the so-called limiting plate, usually have a dark cytoplasm due to a higher concentration of ribonucleic acid. This indicates possibly increased ability to regenerate and that may also hold true for the dark subcapsular cells mentioned.

The recent paper of Post and Rose, to which Dr. Dammin made reference, perturbed us very much. However, we tried to correlate the clinical findings with individual morphologic phenomena, especially with liver cell damage, and not with the entire picture. Moreover, we emphasized that correlation cannot be expected in every individual instance but only by statistical analysis of a large number of cases. One wonders whether such an analysis applied to the material in the paper quoted may not also have yielded some correlation. I would like to stress that we noted lack of correlation between histologic changes and clinical and laboratory findings, especially during the recovery stage. In the instances in which we were able to study multiple biopsy specimens we noted that histologic changes required a much longer period to return to normal than functional alterations. For instance, the serum albumin concentration may have returned to normal, whereas the histologic picture would still reveal marked disturbance. We explained that by the presence of extensive regeneratory changes in the liver which during this period of lag may give a marked distortion of the histologic picture which may not be easily differentiated from liver cell damage.

CLINICOPATHOLOGIC STUDY OF CARDIAC CIRRHOSIS. Paul Kotin (by invitation) and E. M. Hall, Los Angeles, Calif.

*Abstract.* A review of over 7,000 autopsies performed at the Los Angeles County General Hospital during 1942-1946 was undertaken in a clinicopathologic study of cardiac cirrhosis. This was done to establish specific criteria as to the etiologic relationship of cardiac decompensation to cardiac cirrhosis. Sixty-six cases of cardiac cirrhosis were found among 600 cases of decompensation. The duration of decompensation was correlated with the severity of fibrosis. The lesions were placed in one of four classifications depending upon the degree of fibrosis. Age and sex incidence are reported. A few case abstracts are presented showing variations in ages of patients, duration of decompensation and hepatic fibrosis. Cardiac cirrhosis is defined. These livers are usually smaller than normal, dark red or mottled with purplish brown. The surface is often finely granular and they cut with increased resistance. Microscopically, there is often central atrophy of marked degree with a thickened reticular network in the central one-third or one-half of the lobule corresponding with the atrophied areas. In more advanced cases the fibrous network or brush-like fibrous bands occupy progressively greater amounts of the intralobular parenchyma. The central veins are always thickened, at times to a marked degree. The duration of cardiac decompensation varied from 4 to 30 years. Recurrent bouts of decompensation seemed to be most effective in producing hepatic fibrosis.

*Discussion*

(Dr. B. Black-Schaffer, Durham, N.C.) How many of your decompensated patients did not show fibrosis? How many of your decompensated cases which were not eliminated for the reasons you gave did not show it?

(Dr. Otto Saphir, Chicago, Ill.) I think that the changes were beautifully shown here: the severe fibrosis of the liver, the dilatations of the sinusoids, and so on. It is usual to find in cirrhosis of the liver not only the destruction of lobules and the new formation of bile ducts, but also, what seems to me most important, regeneration of liver lobules. I wonder whether in this series of cases you have come across such a true cirrhosis with regeneration and new formation of liver lobules.

(Dr. Russell L. Holman, New Orleans, La.) I thought that I noticed in Dr. Kotin's statistics a disproportionate incidence of cardiac cirrhosis in cases of cardiac failure due to rheumatic fever, and I wonder if he has the feeling that the rheumatic process might influence collagen metabolism in any of the ways that we were discussing yesterday.

(Dr. Hans Popper, Chicago, Ill.) How often were connective tissue bridges noted between the central field and the portal triads? We consider this type of trabeculation of great importance in the distortion of the lobular pattern. This question is also of importance because at present evidence is accumulating that Laennec's cirrhosis starts with centrolobular fibrosis. I should further like to ask whether, in the case presented, actual necrosis of the liver cells was noted in the central zone. We observed it not infrequently in cardiac failure and assumed it to result from associated conditions producing toxic substances such as uremia or myocardial infarction.

(Dr. Kotin) In answer to Dr. Popper's question, in less than 10 per cent of the cases we saw actual hepatic necrosis, but we did not mention that specifically because of the fact that they had a correlated toxic condition which we thought might be responsible for the necrosis. Where the patients died of nontoxic states we did not see any necrosis, but just atrophy.

In reply to the question concerning the connection with the portal triads, in approximately 50 per cent of the cases we thought we saw it in the periportal connective tissue. In those cases we thought we could detect a relationship between the central fibrosis and a later periportal fibrosis. In those cases in which we did see it, we thought the fibrosis in the central part of the lobule was the greater.

In regard to Dr. Holman's remark concerning the disproportion of the group that heart disease represents there may well be a specific relationship between rheumatic fever *per se* and the production of fibrous tissue in the liver. In view of the advancing knowledge of collagenous diseases, we may revise some of our ideas along this line. At present we do not feel rheumatic fever is out of proportion statistically with the incidence of rheumatic fever in all of our autopsy material. If half of our cases showing decomposition had a rheumatic basis, the same ratio was seen in the group presented.

In answer to Dr. Saphir's question, we were surprised at the absence of portal cirrhosis as evidenced by liver cell regeneration, bile duct reduplication, and so on. We did see it in a few cases, but we felt we could not draw any conclusions about it, and perhaps it should have been mentioned in the paper. Thank you for pointing this out. There is some attempt at the formation of a picture not unlike that of portal cirrhosis, but that occurred in 3 cases only, with an associated definite picture of congestive cirrhosis, so we did not mention it.

In reply to Dr. Black-Schaffer, of the 605 cases we eliminated half on the basis of the disease not fulfilling the clinical criteria. These cases were not studied histologically. About 80 per cent of the clinically acceptable cases of chronic cardiac decompensation failed to show findings which we think would be consistent with cardiac cirrhosis.

THE GENERAL PATHOLOGY OF THE DERMATOMYCOSES. Roger Denio Baker, Birmingham, Ala.

*Abstract.* The dermatophyte fungi are less regularly associated with inflammation than fungi causing deep mycoses, for dermatophytes may apparently lie dormant

in the stratum corneum for months, and give rise to lesions only in the presence of unusual moisture and warmth. The dermatophyte fungi are far less prone to produce suppuration, giant cell reaction, and fibrosis. This may be largely a matter of the superficial location of the dermatophytes. They do not seem to be able to persist below the level of the stratum corneum like the organisms causing the deep mycoses. Finally, the dermatophyte fungi are more chronic, in many instances, than any of the deep mycoses. Dermatomycoses persist for decades in some persons. Among the dermatophytoses and saprophytic fungous infections, organisms are readily demonstrated in section in tinea versicolor, erythrasma, tinea unguium, tinea capitis, and tinea favosa, but not so readily in tinea pedis, cruris, and corporis. Fungi are demonstrable in section in essentially all of the deep mycoses except in sporotrichosis. Moniliasis, although not classified strictly as a dermatophyte mycosis, is chiefly a superficial fungous infection affecting skin and mucous membranes. It may produce lesions like those of tinea unguium and tinea corporis with organisms demonstrable in the nails and skin respectively. However, on occasion moniliasis is a serious deep mycosis.

*Discussion*

(Dr. Shields Warren, Boston, Mass.) I am sure all of us who have had biopsy sections of the skin sent in from lesions similar to these have had great trouble in giving any sort of a specific diagnosis. I wonder if Dr. Baker, with his experience in this field, has any suggestions for the practicing pathologists who receive biopsy specimens from these lesions as to the best way of trying to handle them from the histologic angle.

(Dr. Ralph D. Lillie, Bethesda, Md.) Some years ago when we were trying the Bauer chromic acid-Schiff method for the demonstration of glycogen in skin material, we noted with considerable frequency the presence of fungi and yeasts whose capsules stained brilliantly by this method in the corium and on the surface. This method might be of some help in this connection.

(Dr. Baker) As to the technic, the ordinary hematoxylin and eosin method will show, as you know, most fungi. The difficulty of using some special stains is that they tend to stain the keratin too intensely, so that the fungi are obscured. I find the Giemsa stain and methylene blue the most satisfactory, in addition to hematoxylin and eosin. I know of Dr. Lillie's good results with the technic he mentions, but I have not used this method myself.

INFARCTION OF THE PROSTATE. F. K. Mostofi (by invitation) and W. H. Morse, Washington, D.C.

*Abstract.* Among 50 cases of prostatic infarction contributed to the Prostatic Tumor Registry within the past year were 10 with the erroneous diagnosis of carcinoma of the prostate. Prostatic infarction is not infrequently seen in association with nodular hyperplasia of the prostate in patients who have had previous operative interference with the prostate, and occasionally in association with prostatitis. In the early stages there is an area of ischemic necrosis of the tissue with some inflammatory reaction. The peripheral acini are compressed, distorted, and atrophic. Squamous metaplasia soon follows, and in the later stages fibrous replacement of the stroma takes place. Such areas are usually well circumscribed and contain nests of metaplastic squamous epithelial cells. These epithelial nests are entirely benign but have been confused with squamous cell carcinoma of the prostate which is exceedingly rare.

*Discussion*

(Dr. S. Milton Rabson, Fort Wayne, Ind.) What was the status of the epithelium of the posterior urethra in the sections examined?

(Dr. Paul Kimmelstiel, Charlotte, N.C.) We can completely confirm Dr. Mostofi's findings. We have a series of approximately 60 cases recently which we have analyzed, and I would like to add a few remarks. In the first place, we have not been

able to find any direct correlation between the occurrence of infarcts in the prostate and traumatization of the prostate. There were many cases in which no trauma had occurred prior to enucleation of the prostate. In the second place, we have found that true metaplasia, that is, the occurrence of identifiable stratified squamous epithelial cells with intercellular bridges or cornification, is relatively infrequent. The most frequent change is what Dr. Mostofi calls "cells resembling transitional cells." We prefer for these changes the term dysplasia which was first coined by Krompecher, and later taken up by Oberndörfer. That is the most frequent change found, and I think it is worthwhile pointing out that both dysplasia and metaplasia occur frequently in prostates of old men, with or without infarction. However, they do occur invariably around infarcts. There is one last point, namely, that the occurrence of infarcts seems to be well correlated with the size of the prostate. They occur with increasing frequency with increasing size of the prostate in hypertrophy.

(Dr. Robert A. Moore, St. Louis, Mo.) I can add an additional type of information to confirm Dr. Mostofi's conclusions. Some years ago Dr. Mary Lucy Miller was studying dogs' prostates and the effect of hormones on the histologic structure of that organ, using the Carrel-Lindbergh pump. Under these experimental conditions those of you who have used this pump know that the formation of infarcts is frequent in the organ studied. In 3 to 5 days after the prostate is put on that pump the same type of lesion occurs *in vitro* which Dr. Mostofi has described *in vivo*.

(Dr. Mostofi) In this group I saw no change in the epithelium of the posterior urethra.

I wish to thank the other discussers.

CHRONIC "PEPTIC" ULCER: POSSIBILITY OF DIFFERENCES IN PATHOGENESIS. Alvin J. Cox, Jr., San Francisco, Calif.

*Abstract.* Gastric and duodenal ulcers are frequently considered as if they were manifestations of the same disorder, even though differences in sex incidence have been reported and a variety of studies have indicated that gastric hyperacidity is more common in cases of duodenal ulcer than of gastric ulcer. If the background of these two types of ulcer were the same, they should occur together frequently. A survey of 122 cases of chronic or healed duodenal ulcer and 105 of chronic or healed gastric ulcer, discovered in 3,400 successive autopsies, yielded only 13 cases in which both types of ulcer had been present. This is an incidence of only 6 per cent of the cases with ulcer. Yet there were multiple lesions of one or the other of the two types in 36 per cent of the cases. The incidence of associated lesions of the two types is not much greater than the incidence of 3.1 and 3.6 per cent, respectively, for chronic gastric and duodenal ulcer in the entire autopsy series.

Measurements of stomach mucosal area and thickness in 52 cases of chronic or healed ulcer have shown that duodenal ulcer occurred nearly always in association with large stomachs while gastric ulcer was found frequently in small stomachs. The increase in mucosal mass in large stomachs was most conspicuous in the specific secretory portion; this presumably represents a change associated with hypersecretion in patients with duodenal ulcer. It is suggested that this difference in stomachs of ulcer patients, related to the location of the ulcers, may reflect an important difference in the pathogenesis of the two types of chronic ulcer.

*Discussion*

(Dr. Howard C. Hopps, Oklahoma City, Okla.) Is it possible that an obstructive factor in chronic duodenal ulceration might have been responsible for the increased size of the stomach?

(Dr. Ralph E. Miller, Hanover, N.H.) I should like to ask if it is possible that the scarring of the ulcer might account for the decreased size of the stomach.

(Dr. Cox) I think it is impossible to prove that scarring at the pylorus does not influence the size of the stomach. I would not undertake to deny that changes in

muscle mass might result. However, the large mucosal mass in the cases of duodenal ulcer I am not able to account for in this way. Scarring in the stomach itself reduces the area of the mucosa in some cases of gastric ulcer, but many ulcer stomachs are small even though there is very little scarring adjacent to the ulcer, and it is unusual to find chronic gastric ulcer in a large stomach.

HISTOPATHOLOGIC PATTERNS OF BRAIN DAMAGE IN ERYTHROBLASTOSIS OF THE NEWBORN. Kornel Terplan and (by invitation) Sarah Barnes, Buffalo, N.Y.

*Abstract.* Results of histologic analysis of the central nervous system in several cases of erythroblastosis of the newborn are reported. The newborn children had survived 2, 3, 4, and 6 days respectively. One was premature; the others, fullterm. Delivery was not complicated in any case. There were no findings pointing to birth injuries. Only one of them, a non-icteric, was in respiratory distress most of the time; the others, including 2 with distinct icterus, were occasionally slightly cyanotic. Their deaths seemed all to be caused by regurgitation and aspiration of food and by extensive hemorrhages in the lungs causing considerable consolidation of the alveolar parenchyma. The gross findings in the central nervous system varied. In some there was very marked swelling and hyperemia of the brain with the borders between gray and white matter considerably blurred, but without icterus. In others, there was anemia of the brain with typical kernicterus. A third type showed anemia and edema of the brain, icteric plexus, and icteric spinal fluid, but no kernicterus.

Microscopically, extreme anoxic cortical brain damage was found affecting in various degrees the entire pallium. In numerous gyri most of the nerve cell layers had disappeared. In sections stained by the Nissl method the characteristic break in the nerve cell bands could be demonstrated readily in most cortical fields. As so frequently seen in anoxic brain damage, the deeper convolutions showed the more intense and diffuse changes. In the brain sections of the 6-day-old child, the architecture was even more completely obliterated. Proliferating capillaries, here and there recent capillary hemorrhages, and intense microglial reaction took the place of the vanished nerve cells. In contrast to these extreme cortical changes in the cerebrum, the cerebellum and the nuclei of the brain stem, thalamus, and hypothalamic region showed comparatively little damage, including the nuclei in which (in one case) kernicterus was observed. Characteristically, the striatum, and to some extent Ammon's horn, took part in this severe cortical damage. Another pattern seen in a few cases surviving 1 to 3 days showed multiple capillary hemorrhages in the deep and subcortical white matter of the cerebrum, but only little architectural derangement of the cortex.

The findings presented followed largely the pattern observed in known types of anoxic brain damage, varying only in degree. In 2 of our cases they were as extreme as in insulin shock and in some cases of death from anesthesia.

*Discussion*

(Dr. Israel Davidsohn, Chicago, Ill.) I should like to ask if these changes were ever found in stillborn babies with definite erythroblastosis, and while Dr. Terplan did not speak of kernicterus, I would like to know whether he has observed kernicterus in stillborn babies. He made reference to the fact that there were no thrombi in the blood vessels. In view of the fact that some writers have attributed much significance in these cases to the finding of agglutinated red cells, I would like to know whether Dr. Terplan excluded agglutinated red cells when he spoke of thrombi. Furthermore, Dr. Terplan mentioned that all these babies had transfusions. I should like to know whether some of them had so-called replacement transfusions, and whether there was any relationship between the amount of blood given and the occurrence of these changes.

(Dr. James F. Rinehart, San Francisco, Calif.) I want to ask if Dr. Terplan has examined the adrenal glands systematically in this condition. Several years ago we

observed that there was a very frequent occurrence of severe cortical degenerative changes in a high proportion of infants with erythroblastosis, and I wonder if this factor might be associated with the changes shown here.

(Dr. Harold D. Palmer, Denver, Colo.) May I ask what stain was used in these sections?

(Dr. Terplan) In answer to Dr. Davidsohn, I wish to say that I have not seen any cortical damage in stillborn babies, nor did I see kernicterus in the stillborn. I have observed kernicterus in erythroblastotic children dying 24 hours after birth. Usually kernicterus is much more pronounced after the second day. In some erythroblastotic children with extensive generalized icterus, no kernicterus is seen at all, even in the presence of markedly icteric spinal fluid and plexus; or, we have seen kernicterus localized to a few nuclei in the brain stem but absent in others.

In all of the cases from which pictures were shown in this presentation, transfusions had been given, usually varying in amounts from 50 to 60 cc. Cases with exchange transfusions were not included in this presentation, as most of this material is of recent date, when exchange transfusions became more popular. It is not completely worked up as yet.

As to the question regarding thrombi and agglutination of red cells: In a few cases in which destruction of the cortical tissue is quite extreme, there is considerable engorgement of some of the subcortical veins. One is under the impression that there is stasis in these veins. The findings, however, are not conditioned by thrombosis of the capillaries or veins. The histologic picture as presented, in particular in the last 2 cases, is in no way specific for erythroblastosis. It is exactly the same as we found in various conditions of anoxic brain damage—severe hypoglycemic brain swelling, in prolonged shock, in some cases of burns, oleum chenopodium poisoning and certain types of death from anesthesia. In fact, in one case of hemorrhagic disease of the newborn in which known incompatibility of blood factors was not present, a very similar change was seen in the absence of thrombosis of the veins. Here, too, I did not see agglutination of cells.

(Dr. Davidsohn) Have you observed intravascular agglutinates of red cells?

(Dr. Terplan) I have not.

In answer to Dr. Rinehart, I do not recall exactly our figures with regard to adrenal damage in erythroblastosis. In some cases, I recall, there were capillary hemorrhages in the medulla. I have no recollection of extensive damage to the cortex, but I would have to recheck my material to give a more definite answer to Dr. Rinehart's question. In most of our cases all organs were studied. As I wanted to speak only of brain damage, I did not bring along the figures regarding findings in other organs.

The stain used in this study, and from which the photographs were taken, was the Nissl method. Other routine stains were used. Those who want to prevent as much shrinkage as possible in the unusually soft brain tissue of the neonatal period should disregard the conservative method for dehydration. We proceeded very gradually after fixation in formaldehyde. In fact, we used first between 20 and 30 per cent alcohol, and moved only gradually through various dilutions until the material was finally placed in 95 per cent alcohol.

A Fibrous Component of the Nerve Axon Demonstrated in Thin Sections with the Electron Microscope. Betty B. Geren, Cambridge, Mass.

*Abstract.* A study of normal nerve (squid, frog, rat, and human) with the electron microscope, using thin sections, was presented. The demonstration of an axonal filamentous component (100 to 200 Å in width) corroborates earlier findings in fragmented invertebrate nerves. Dense-edged fibrils termed "neurotubules," previously described in preparations of fragmented nerves, are not found in the axon. In the few instances in which they have been observed in thin sections of nerves they have appeared in the connective tissue sheath.

THE MECHANISM OF THE ANAPHYLACTOID PHENOMENON. Paul Gross and (by invitation) Jack H. U. Brown, Pittsburgh, Pa.

*Abstract.* Hanzlik and Karsner characterized the anatomical findings of the anaphylactoid phenomenon by the following: (1) pulmonary distention; (2) peribronchial edema; (3) pulmonary congestion and hemorrhage; (4) constrictive arterial beading; (5) thrombi in pulmonary vessels; and (6) cardiac dilatation. Not all of these features were found constantly. In some animals the respiratory system was chiefly involved, and in others chiefly the circulatory system. In the light of our present concepts all except the last two of the above criteria are non-specific because they are manifestations of shock, regardless of origin.

The following experiments were designed to explain the genesis of pulmonary thrombosis and the cardiac dilatation following intravenous injection of particulate matter. The lethality of particulate matter is a direct function of the size. In a series of 60 animals, particles above 0.6 $\mu$ killed none of the animals; particles between 0.6 and 0.2 $\mu$ killed increasing percentages of animals injected intravenously, and particles below 0.2 $\mu$ killed all animals tested. This relationship is true regardless of the type of material tested. In our experiments carbon, aluminum oxide, ferric oxide, antimony trioxide, silica, and clay have been used. The lethality of a particular size of material can be correlated with the clumping of the material in the presence of plasma; the amount and type of aggregate formed being a function of the particle size. The lethality also can be correlated with the amount of protein adsorbed from plasma. Those sizes which do not kill adsorb 4 to 6 mg. of protein nitrogen per 15 mg. of material, while those sizes producing high mortality adsorb up to 30 mg. of protein nitrogen.

The validity of the concept has been tested by a simple procedure. Cannulas were placed in the pulmonary arteries of rabbits and the effective perfusion pressure was measured during the perfusion of the lungs with saline solution, plasma, and plasma plus particulate matter. When particulate material was added to the perfusion mixture, the effective perfusion pressure was nearly doubled, indicating massive blockage of capillaries and smaller vessels. This finding was confirmed by histologic sections. The sections obtained by experimental means were similar to those obtained from animals killed by intravenous injection of particulate matter.

In summary, the mechanism of action of the anaphylactoid phenomenon appears to be the formation of aggregates of particulate matter with subsequent blockage of pulmonary vessels. The aggregation of the material is due to the adsorption of plasma proteins and the severity of the reaction is a function of decreasing particle size.

*Discussion*

(Dr. Howard C. Hopps, Oklahoma City, Okla.) Twenty-five years or so ago Northrup and collaborators at the Rockefeller Institute made some very extensive studies showing that fine particulate matter injected intravenously, and even blood plasma after certain manipulations injected intravenously, would kill very quickly. I think their conclusions were to the effect that no embolic phenomenon occurs. I wonder if Dr. Gross is familiar with their work, and if he can correlate it with his observations.

(Dr. John W. Harman, Madison, Wis.) This phenomenon is very interesting and the results extremely well observed. It is unquestionable that there has been agglutination of these clay particles. However, it is very challenging because in electrophoretic work the particles are coated with the protein and are usually repellent because of the same charge. The breaking-down of the fat in the blood and the coating of it with protein has the same result. The fact that the observer was able to prepare a suspension and keep it in that state before injecting it further indicates that he may have implicated another factor. I wonder if Dr. Gross will elucidate his thoughts on that particular additional factor.

(Dr. Gross) I am sorry, but I am not familiar with the work from the Rockefeller Institute on the injection of plasma and the inability of that group to demonstrate embolic phenomena. However, as noted early in the presentation, Hanzlik and Karsner repeatedly noted intravascular occlusions and found thrombi, conglutinations of platelets or red cells.

In regard to the matter of the electrophoretic observations and the repulsion of coated particles in the electrophoretic experiments, I have no explanation for that particular phenomenon, but in a recent paper by M. H. Knisely, E. H. Bloch, F. Brooks, and L. Warner concerning the sludging of blood, a pertinent *in vivo* observation was made. Particles and cells were observed to become coated and adhesive. There might, therefore, be a difference in behavior of the coated particles *in vivo* and under the circumstances of the electrophoretic experiment.

In regard to the nature of the suspension, it has been very troublesome for us to produce stable suspensions of finely divided particulate matter. However, the method of preparation of such stable suspensions is the subject of a paper by Dr. Brown and associates at present in press in The American Journal of Public Health.

EOSINOPHILIC MENINGITIS: TWO CASE REPORTS. R. L. Ferguson and (by invitation) C. B. McVay and John Hill, Vermillion, S.D., and Yankton, S.D.

*Abstract.* A 52-year-old farmer entered the hospital complaining of severe headaches. His past history was negative except for severe photophobia and persistent headaches. The spinal fluid contained 360 cells per cmm., 78 per cent eosinophils. The white cell count was 16,000 with 4 per cent eosinophils and 83 per cent polymorphonuclear leukocytes. Unsuccessful attempts were made to isolate organisms from the spinal fluid. Blood cultures were negative. Penicillin and sulfadiazine were administered to the patient. On the fifth day the patient became cyanotic and died after several convulsions. The meningeal vessels were dilated. A pale gray, granular pseudo-exudate was found on the meninges. It was fairly well distributed over the frontal and parietal regions, extending into the posterior and middle fossae with a slight amount in the anterior fossa. Histologically, the leptomeninges showed many eosinophils and a few lymphocytes embedded in a small amount of fibrin. Special stains failed to reveal bacteria or other parasites.

A 25-year-old white housewife was admitted to the hospital in coma. Her past history was negative except for a cold 2 days previous to entry. There were numerous petechial hemorrhages over the body. The spinal fluid revealed a cell count of 5,480 per cmm. with 92 per cent eosinophils. The white cell count was 19,300; no eosinophils were noted in the differential count. Several unsuccessful attempts were made to isolate an organism from the blood and spinal fluid. The patient received penicillin, streptomycin, and sulfadiazine. She responded readily to medication and was discharged 9 days after hospital entrance. Since this patient recovered from rather specific treatment, it would appear that bacteria may have been the causative agent, even though it was impossible to isolate the organism.

*Discussion*

(Dr. P. O'B. Montgomery, Dallas, Texas) We saw a similar case and I was unable to find any reports in the literature of such cases with eosinophils in the spinal fluid, but they certainly occur in parasitic infestation of the brain. Our patient was a 40-year-old male who had chronic meningitis of low grade for 3 weeks before he entered the hospital; he stayed in the hospital and had a temperature of 101° or 102° for 2 weeks, and then spontaneously recovered and has remained well since. No one in that area had had the disease. All bacteriologic examinations of the spinal fluid and examinations for fungi and parasitic infestation were negative.

(Dr. Walter H. Sheldon, Atlanta, Ga.) We are studying at present a very similar

case of a young adult Negro male who died in the emergency clinic of our hospital. The family gave a history of illness of about 1 week's duration, but the patient had not been seen by a doctor. Autopsy showed marked and diffuse meningitis, chiefly about the base of the brain. The prosector suspected tuberculous meningitis, but on close examination of the other organs no evidence of active tuberculosis was encountered. Histologic study of the brain revealed a picture very similar to that shown here. In addition, it showed an extensive involvement of the choroid plexus on both sides, and many small focal areas of recent softening located in the basal ganglia. We also found a slight but distinct interstitial myocarditis which could not, on a morphologic basis, be classified under the catch-all term of Fiedler's myocarditis. Cultures of this particular case have shown nothing. Those for acid-fast bacilli have not yet been reported, but I feel quite confident from the morphologic picture that they will show nothing.

CYSTINOSIS: A REPORT OF TWO CASES WITH POST-MORTEM EXAMINATION. B. Earl Clarke and (by invitation) Herbert F. Jackson, New York, N.Y.

*Abstract.* Cystinosis, or Lignac's disease, is a rare familial disturbance of protein metabolism occurring in infants and young children. It is characterized clinically by polydipsia and polyuria, anorexia, arrested growth, and, finally, renal failure. Pathologically, there is marked deposition of crystalline cystine in various organs and tissues and a peculiar interstitial nephritis and glomerulonephrosis. So nearly as we can determine, only 14 post-mortem examinations have been published, none of which were in the United States. If American pediatricians and pathologists become familiar with the condition, no doubt other cases will be recorded.

*Discussion*

(Dr. Shields Warren, Boston, Mass.) Was there anything in the family history that would indicate that other cases had occurred in earlier generations?

(Dr. Clarke) We tried to investigate the familial aspects of these cases. We have studied the father, mother, and a normal sister, and found no increased cystine in their urine. We had some difficulty getting the family's history; the mother tells a story about the father having a brother who had some children who died under peculiar circumstances, but the husband was very much upset and refused to talk or let us investigate.

THE FATE OF TRANSFUSED PLASMA PROTEIN, STUDIED WITH PLASMA LABELED WITH RADIOACTIVE SULFUR. L. J. Zeldis and S. C. Madden, Upton, N.Y.

*Abstract.* Blood plasma was labeled with radioactive sulfur ($S^{35}$) by including synthetic *dl*-methionine containing $S^{35}$ in the diet of a normal dog. This labeled plasma had most of its radioactivity in the cystine sulfur fraction of its proteins, less in the methionine sulfur, the ratio being about 3:2. It was given to 4 6-months-old dogs, intravenously to 2, orally to 2. Twenty-four hours later the animals were perfused with saline solution under ether anesthesia and organs and tissues were frozen. Sulfur chemical partition and sulfur radioactivity of the organ and tissue total proteins (tri-chloroacetic acid-precipitated, lipid-extracted residues) were estimated. Of the injected plasma sulfur, 4.8 per cent was excreted in the urine; of the fed sulfur, 8.6 per cent. Twenty-five per cent of the injected sulfur, as compared to 4 per cent of the fed sulfur, was present in the circulating plasma proteins after 24 hours. The proteins of the intestinal mucosa and pancreas contained 2 to 5 times as much labeled sulfur after feeding as after injecting the labeled plasma. The proteins of liver, kidney, lymph node and muscle were slightly more enriched after feeding the labeled plasma than after its injection. About 62 per cent of the plasma protein sulfur circulating at the time of injection of the labeled plasma had been replaced at the end of 24 hours, apparently largely by the normal extravascular "plasma" protein and partly by newly formed plasma protein. The amount of circulating

plasma protein produced from the fed plasma protein during the 24-hour labeled period approximated 2 per cent of the total circulating plasma protein. The significance of these data is discussed.

*Discussion*

(Dr. Russell L. Holman, New Orleans, La.) I think Dr. Madden and Dr. Zeldis are to be congratulated on the progress that they have made in this work. I would like to ask Dr. Madden about the rate of disappearance of radioactive sulfur from the blood stream in the first 24-hour period.

(Dr. Lee N. Foster, Indianapolis, Ind.) Was fat tissue examined for the presence of radioactive sulfur?

(Dr. Madden) It is easy for us to answer both of those questions; we do not know. We have not examined fat tissue and we do not know about the rate of disappearance during the first 24 hours except as it is indicated by the 24-hour rate and by the actual observations along such curves as we have reported previously with $N^{15}$-labeled lysine in plasma protein; and that Miller, Yuile, Whipple, and associates have confirmed with $C^{14}$-labeled plasma protein lysine.

ENDOPHLEBOHYPERTROPHY AND PHLEBOSCLEROSIS. Maurice Lev and Otto Saphir, Chicago, Ill.

*Abstract.* A process of endophlebohypertrophy was found to occur in various veins from birth, at the mouths of tributaries, adjacent to bone and muscle, and at a linear region adjacent to the accompanying artery. The process is typified grossly by longitudinal streaks and plaques. In youth these plaques are relatively smooth and white. Histologically, the plaques consist of a proliferation in the direction of blood flow of elastic tissue, muscle, and collagenous connective tissue of the intima, and of the intimal aspect of the media, accompanied by the basement membranes around the muscle and elastic tissues.

These plaques of endophlebohypertrophy undergo changes with age. Grossly, they become broader, deeper, firmer, more yellow, more "geographic," and fenestrated. Histologically, corresponding to those gross alterations, retrogressive changes are found, which are included under the term endophlebosclerosis. These changes are: basophilic staining, vacuolization, and lacunar formation of the ground substance, believed to be due to depolymerization and dissolution of glycoproteins; disruption and loss of elastic fibers; loss of muscle cells; fragmentation of the internal elastic lamella; and replacement by fibrous connective tissue. Changes in the reticular and glycoprotein components of the basement membranes accompany the changes in the elastic and muscle tissues. The endothelium does not share in the process of hypertrophy. The media initially shows hyperplasia of its elements, but subsequently becomes excavated, with a relative increase in connective tissue. This process has been seen in all large veins studied—popliteal, posterior tibial, femoral, iliacs, inferior and superior venae cavae, innominate, portal, splenic, hepatic, pulmonary, and renal. It is more marked in the veins of the lower extremities, the iliacs and inferior vena cava. In cases with markedly increased venous pressure these processes are much more diffuse. These result grossly in a diffuse whitening of the pulmonary veins in instances of left ventricular failure, and of the hepatic, inferior vena cava, and iliac veins in right ventricular failure. In addition, the iliac veins disclose in instances of back pressure an increase in thickness of the media. (The media has not yet been studied in the other veins.)

It is suggested that local forms of mechanical stress, and the generalized increase in venous pressure have a common denominator in their action, and hence there is a common denominator in the reaction of the vein; namely, endophlebohypertrophy and endophlebosclerosis. This action is normally localized at points of physiologic stress. In cases with increased venous pressure this action is widespread, and more severe. In addition, increased venous pressure exerts a lateral force resulting in hypertrophy of the media.

*Discussion*

(Dr. James F. Rinehart, San Francisco, Calif.) Certain of the basic reactions here bear much resemblance to the fundamental reaction seen in the arteriosclerotic process, I believe. I wonder if Dr. Lev has correlated the association of arteriosclerosis and this lesion.

(Dr. J. M. Ravid, New York, N.Y.) I should like to ask if any studies were made on the central vein of the adrenal gland and what is the incidence in the author's statistics of the hypertrophy of this vein.

(Dr. Lev) In answer to Dr. Rinehart's question, we have seen the same process in the arteries. The process in the arteries is now being studied by Drs. Learner, Catchpole, and myself at the University of Illinois. We are not yet ready to make any positive statement as to the nature of this process in the arteries. There is no reason to believe that the process here is basically much different from that in the vein. Our method of procedure is to first determine the functional anatomy of the arteries and their changes with age. We are not assuming anything, but are starting from scratch. We believe that is the way to solve the problem in the arteries, as we think we are solving it in the veins.

In reply to the question concerning the adrenal vein, this is now being studied, and we have as yet nothing to offer.

(Dr. Saphir) In regard to Dr. Rinehart's question, fatty changes are very important in certain stages of arteriosclerosis. We have practically never found fat in these cases of endophlebohypertrophy.

ENDOCARDIAL FIBROELASTOSIS: A STUDY OF EIGHT CASES.* John T. Prior (by invitation), J. Howard Ferguson and (by invitation) Tyree C. Wyatt, Syracuse, N.Y.

*Abstract.* An analysis of the clinical, gross and microscopic features of 8 cases of endocardial fibroelastosis is presented. This unusual type of heart disease, generally thought to be due to an inborn derangement of elastic tissue, is most commonly seen in infants dying within the first year of life. The average age at death in this series was 4 months, excluding one stillborn at 5 months of gestation. The extremely short duration of life after the onset of symptoms and the fact that most of the children had appeared normal at birth were striking clinical characteristics. Cyanosis was the most frequent presenting sign, although dyspnea and anorexia were nearly always noted.

The anatomical lesion consisted of a yellowish white, thickened endocardium and involved the left side of the heart only. Left ventricular hypertrophy, giving the heart a characteristic globular shape, was invariably present. Upon microscopic examination the endocardium was seen to be composed entirely of delicate elastic fibers and only that portion of the myocardium forming the papillary muscles revealed fibrosis and calcification. Valvular lesions noted in 3 of the cases also were confined to the left side. The affected cusps and leaflets, which were swollen, nodular, and glistening white, demonstrated very minute amounts of elastic tissue. The swelling in the valves was due to a proportionate increase in the normal valvular tissue with occasional condensation of reticular fibers. Since there was no evidence of an inflammatory process, either within the endocardium or the valves, the term "endocardial dysplasia" is suggested to replace the inaccurate and misleading "fetal endocarditis."

The characteristics of elastic tissue in general and the various theories of the pathogenesis of endocardial fibroelastosis are reviewed. Additional mechanisms are postulated to explain the mechanism of the left ventricular failure which was the cause of death.

*Discussion*

(Dr. Hilliard Cohen, Kansas City, Mo.) I should like to know whether any

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

abnormalities in the coronary circulation were noted. I refer particularly to an origin of the coronary arteries from the pulmonary artery.

(Dr. Robert Fienberg, Framingham, Mass.) On Thursday I reported a case of endocardial sclerosis of the right and left ventricles in a World War II veteran before the International Association of Medical Museums, and I must say that the adult type probably has nothing to do with the fetal type. For one thing, the absence of cases in puberty and adolescence is striking. This indicates that any degree of fetal endocardial sclerosis leading to a constrictive endocarditis is incompatible with life. Another interesting thing is that all the cases of adult endocardial sclerosis which have been reported, and most of the cases have appeared in the German and Swiss literature, have been thought to be due to an inflammatory, toxic, or sensitization phenomenon, while most of the authors reporting the fetal type have believed that the lesion is a developmental defect. In both forms, however, there is an increase in elastic and fibrous tissue, so that fundamentally both can be called fibroelastosis. If we are going to use that term, we should use a qualifying adjective, such as congenital, fetal, or adult. In some of these adult cases, eosinophilia has been reported. This eosinophilia may disappear at the end of the disease.

(Dr. Paul Kimmelstiel, Charlotte, N.C.) As I understand it, fibroelastosis is not necessarily connected with valvular deficiencies. I would like Dr. Prior to make some comments on the marked hypertrophy of the left side of the heart which he demonstrated in all of the cases. How does he account for that?

(Dr. Otto Saphir, Chicago, Ill.) I should like to know whether the authors also observed circumscribed areas of fetal fibroelastosis in the heart. Here they have shown diffuse changes in the endocardium. I have seen a number of cases in which changes were found in localized areas. Was any dilatation of vessels seen within the myocardium? This is asked because of the possibility that fibroelastosis has closed off a number of openings of thebesian vessels. I also wonder whether there are changes in the myocardium secondary to the closing of these vessels.

(Dr. Peter Gruenwald, Brooklyn, N.Y.) I noticed in several cases a rather brief terminal history of dyspnea, cyanosis, and in one case, gurgling cough. I wonder whether this could be caused by a peculiar mononuclear pneumonia which is frequently found with congenital heart disease and is morphologically identical with that found in infants who die suddenly at home.

(Dr. Tobias Weinberg, Baltimore, Md.) Dr. Prior has noted the occurrence of valvular involvement together with the involvement of the mural endocardium, but no involvement of the valves alone. As for the occurrence of the valvular deformity without the coexistent involvement of the mural endocardium, we have one such case in our series of 12 cases of diffuse endocardial fibroelastosis, and Craig in his analysis of the cases at the Children's Medical Center in Boston reports 6 cases in which only the valves were involved.

Concerning focal involvement of the mural endocardium—if I may be permitted to answer Dr. Saphir's question directed to Dr. Prior—in our series of 421 autopsies in children up to the age of 10 years, there are 91 cases in which there is focal endocardial fibroelastosis involving the mural endocardium of either the right or left ventricles or both. In most of these cases the hearts were grossly normal otherwise.

(Dr. Prior) In answer to Dr. Cohen's question regarding the coronary artery defects, we studied these vessels thoroughly, not only the coronary vessels, but those of the entire body, looking for some change; we have found none, and this includes the large and small vessels.

I am very glad to hear about the adult cases. The only case I was able to find in my search, and I have been rather unsuccessful, was the one described by Comeau.

In reply to Dr. Kimmelstiel's question regarding myocardial hypertrophy, the etiology of myocardial hypertrophy is easy to explain when we have valvular abnormalities, but in the absence of valvular changes, I have no idea why it occurs.

In reply to Dr. Saphir's question, we found no focal changes in the myocardium other than in the particular portion of the myocardium which went to form the papillary muscles, and here there was sometimes hydropic degeneration, fibrosis, and calcification, but this latter was seen in only about half the cases.

The lung changes consisted of pulmonary edema, and we found no evidence in any of the cases of anything that could positively be considered an early pneumonic process, such as Dr. Gruenwald mentioned.

In answer to Dr. Weinberg, I was not aware that such conditions as he described, that is, the valvular changes without endocardial pathology, did occur. There have been instances where this condition has occurred associated with other congenital abnormalities, patent ductus and sometimes interventricular septum, but I did not find such anomalies in our cases.

OBSERVATIONS ON ARTERIOVENOUS COMMUNICATIONS ("HEMANGIOMA") IN THE LUNG. Averill A. Liebow and (by invitation) Harvey Kausel, Gustaf E. Lindskog, and Arnold H. Janzen, New Haven, Conn.

*Abstract.* Surgical specimens of the involved lobes from 3 patients with clinically demonstrated large arteriovenous communications were prepared as vinylite bronchovascular casts. The anatomical findings were correlated with clinical data regarding the magnitude of these shunts obtained by preoperative cardiac catheterization and blood gas analyses. Anatomically there was variation in the arrangement of the communicating vessels, but all had in common at least one large saccular channel that brought at least one large artery and vein into direct communication. Smaller contributions from other vessels were usually present also. The sacs themselves in one instance were the source of subsidiary venous branches. It was possible to inject the bronchial artery in one specimen. This was slightly enlarged, presumably on account of its function as a source of the vasa vasorum, but it did not directly participate in the formation of the lesion.

MORPHOLOGIC EVIDENCE OF VASCULAR INJURY AS A POSSIBLE MECHANISM OF SUDDEN DEATH DURING INFANCY. Jacob Werne and (by invitation) Irene Garrow, Jamaica, N.Y.

*Abstract.* Histologic and epidemiologic evidence has previously been reported indicating that the sudden death of apparently healthy infants is usually associated with respiratory disease. Because such cases are not routinely subjected to complete pathologic study, they are ordinarily certified in most medicolegal jurisdictions of this country as accidental mechanical suffocation. During 1947, 1,663 such certifications appeared in the U.S. mortality tables (for infants under 1 year). The present report of our continued study of this problem is based upon the findings in a consecutive series of 200 infants found dead while in apparent health, in whom the gross autopsy findings were inadequate to explain death. In addition to the evidence of respiratory disease, microscopic study disclosed widespread vascular engorgement, vascular thromboses, mural vascular and tissue edema, perivascular hemorrhage, and focal tissue reactions mainly in the form of mononuclear cellular infiltration. Such changes were not found in control cases of homicidal and animal experimental mechanical suffocation. Analogous changes were seen in a series of infants observed to die of fulminating respiratory and other infections of known etiology. It is suggested that the presence of these vascular alterations and their effects represent a possible mechanism for the unexpected death in such cases.

*Discussion*

(Dr. Kornel Terplan, Buffalo, N.Y.) To some extent I can confirm Dr. Werne's observations, especially in regard to the findings in the upper respiratory tract. In those cases in which very fulminating infections were produced by *Haemophilus influenzae,* we were able to recover, by cultural methods, the influenza bacillus post

mortem from the heart blood and sometimes even from the spinal fluid in the absence of any gross evidence of meningitis. It is my impression that in most of these cases there is a true septicemia.

(Dr. Werne) I wish it were true that we could recover pathogenic organisms in every one of the instances of sudden death presented. In many cases with the best bacteriologic technical facilities available, we do not recover significant organisms from post mortem blood or viscera. We have the impression that the etiology of the fulminating infections that cause these deaths is probably other than simply bacterial.

(Dr. Terplan) I meant to refer only to infections by *Haemophilus influenzae*.

PULMONARY ARTERIOSCLEROSIS. T. C. Laipply and (by invitation) C. I. Fisher, Chicago, Ill.

*Abstract.* A new classification of pulmonary arteriosclerosis is presented. This is based on the presence or absence of cor pulmonale, or chronic pulmonary disease, or chronic cardiac disease, with sclerosis of the pulmonary arteries and arterioles. It is emphasized that the exact cause of arteriosclerosis in human beings has not been established with certainty. The confusion resulting from the use of the terms primary and secondary pulmonary arteriosclerosis seems sufficient to warrant their being discarded. One case of pulmonary arteriosclerosis with cor pulmonale and without chronic cardiac or pulmonary disease, and 3 instances of pulmonary arteriosclerosis associated with cor pulmonale and chronic pulmonary disease are presented. The most common symptoms were dyspnea, cyanosis, cough, and weakness. The most significant signs were hepatomegaly, electrocardiographic evidence of right heart strain, as well as right axis deviation, and roentgenographic evidence of an enlarged pulsating pulmonary conus. Polycythemia was not a striking sign. The present concept of Ayerza's disease is discussed. The conflicting ideas as to its exact meaning make it desirable to discontinue the use of the term "Ayerza's disease."

*Discussion*

(Dr. Hans Popper, Chicago, Ill.) In the cases without primary abnormalities in heart and lung, was focal thinning of the muscular layer in the branches of the pulmonary artery noted? Such congenital aberrations have been suggested as the cause of primary pulmonary sclerosis.

(Dr. Benjamin Castleman, Boston, Mass.) I wonder whether the authors examined the deep leg veins to see whether some of the cases might have been embolic rather than primary disease of the pulmonary vessels. Also was there any history of an operation that might have led to the formation of thrombosis of the leg veins?

(Dr. David M. Spain, Valhalla, N.Y.) I think it should be stated that since the advent of catheterization of the pulmonary artery it has been demonstrated that pulmonary hypertension may exist without any hypertrophy of the right ventricle. In view of that I do not think that the absence of right ventricle hypertrophy is sufficient to rule out pulmonary hypertension as a cause of vascular sclerosis.

(Dr. Laipply) In answer to the question about thinning of the muscle walls, we did not note significant thinning of the muscular layers of the vessels in the absence of marked intimal arteriosclerosis. When intimal sclerosis was marked, sometimes thinning of muscularis was present.

As for the deep veins, they were not examined any more than in routine autopsies where opening of the iliac veins was done, but we did not find any evidence of emboli.

THE INFLUENCE OF CORONARY ATHEROSCLEROSIS AND MYOCARDIAL HYPERTROPHY ON THE FLOW-CAPACITY OF THE CORONARY CIRCULATION AS DETERMINED POST MORTEM. Theodore Robertson (by invitation), New York, N.Y.

*Abstract.* The status of the coronary vascular system has been investigated in more than 40 normal and diseased hearts by means of a combined perfusion-injection apparatus, adapted by Dr. A. T. Ladd from materials and methods previously em-

ployed by Dock, Prinzmetal, and Salans and Tweed. Quantitative perfusion of the coronary system with kerosene is followed by injection of the arteries with radio-opaque latex. Dissection and radiography are performed by the methods of Schlesinger. In each case the flow-capacity of the coronary system is recorded in cubic centimeters of kerosene per gram of heart per minute, as determined by observing the actual flow through the apparatus and coronaries under a perfusion pressure of 150 mm. of Hg and calculating the actual flow-capacity by means of flow-pressure-resistance formulae. The figure thus obtained proves reproducible within ± 10 per cent when repeated observations are made on a single specimen.

Under the standardized conditions the flow-capacity of 7 normal adult hearts ranged from 4.0 to 8.2 cc. per gm. per min. (average 5.5). In 7 hearts showing myocardial hypertrophy (weights ranging from 340 gm. in a young woman with hypertension for 4 years, to 874 gm. in a case of aortic insufficiency, average 615 gm.), without significant coronary sclerosis, the flow-capacity ranged from 1.0 to 5.4 cc. (average 2.9). In 4 hearts exhibiting severe coronary sclerosis without coronary occlusion, myocardial infarction, or hypertrophy, the range of flow-capacity figures was 2.1 to 4.8 cc. (average 3.5). Hypertrophy and coronary sclerosis were present together in many diseased hearts and this was true in all 5 cases with myocardial infarction. In the 7 hypertrophied hearts having severe sclerosis without infarction the average weight was 467 gm. and the average flow-capacity figure was 2.4 cc. (range 1.2 to 2.7). In the 5 hearts that exhibited myocardial infarction in addition to hypertrophy, the average weight was 576 gm. (range 370 to 628) and the average flow-capacity 1.6 cc. per gm. per min. (range 1.4 to 2.1). All of these patients suffered from angina pectoris during life. Hearts from 3 other cases with angina pectoris have been studied. One heart was hypertrophied and exhibited severe coronary sclerosis without occlusion or myocardial infarction; the flow-capacity was 2.6 cc. per gm. per min. The other 2 cases were instances of severe aortic insufficiency with marked hypertrophy (620 and 874 gm.) and little coronary sclerosis. The flow-capacity was 3.6 and 5.4 cc. per gm. per min., respectively. The data substantiate the hypothesis that angina pectoris results from relative myocardial ischemia.

Considered together, the findings suggest that the hemodynamic influences of myocardial hypertrophy and coronary atherosclerosis are complementary and that hypertrophy alone often has a significant effect. They support the observations of Roberts and Wearn (*Am. Heart J.*, 1941, **21**, 617–633) that during hypertrophy the cross-sectional diameter of the coronary bed does not keep pace with the increase in muscle mass, and they extend the perfusion studies of Dock (*J. Exper. Med.*, 1941, **74**, 177–186), which indicated that the flow-capacity diminishes during normal growth and hypertrophy.

SERUM PHOSPHOLIPIDS IN EXPERIMENTAL ATHEROSCLEROSIS. Aaron Kellner, New York, N.Y.

*Abstract.* Rabbits fed on a high cholesterol diet for long periods were found to have a reversal of the normal relationship between serum phospholipid and cholesterol. The blood serum of normal rabbits usually has a slightly higher content of phospholipid than of cholesterol. The cholesterol-fed rabbits were found to have serum total cholesterol levels that far exceeded their serum phospholipid levels. This relative increase of cholesterol with respect to phospholipid was present invariably in animals that developed atherosclerosis. A similar alteration in the cholesterol-phospholipid ratio has been observed in dogs and in chicks that developed atherosclerosis following cholesterol feeding. The stability of the serum lipid emulsion depends upon the phospholipids (Boyd, Ahrens, and Kunkel), and upon the alpha and beta lipoproteins (Gurd). An increase in the blood cholesterol without a concomitant increase in phospholipid results in the aggregation of lipids into particles

of larger size causing the serum to appear milky or "lipemic," and in a less stable lipid emulsion from which the lipids are more apt to settle out. This alteration in the physicochemical state of the blood lipids appears to facilitate the deposition of lipids within the walls of the blood vessels and may be an important factor in the pathogenesis of experimental atherosclerosis.

In another group of experiments it was found possible to produce striking elevations of the blood phospholipid content of rabbits by the intravenous injection of the synthetic detergents tween 80 and triton A-20. This phenomenon was applied to the study of the rôle of serum phospholipids in the development of experimental atherosclerosis. Groups of rabbits were fed a high cholesterol diet and given repeated intravenous injections of tween 80 and triton A-20 for periods of 9 to 12 weeks. Suitable control animals were fed the same cholesterol diet but received no intravenous detergents. The rabbits that were injected with detergents had high blood cholesterol and high blood phospholipid levels, and had significantly less atherosclerosis of the aorta than did the control animals with high blood cholesterol levels but considerably lower blood phospholipid levels. It was concluded from these experiments that the level of blood cholesterol *per se* was not the sole determinant in the pathogenesis of atherosclerosis. A relative decrease in phospholipid was regularly observed in the animals developing atherosclerosis. The serum phospholipids, if present in sufficient quantity, appear to stabilize the serum lipid emulsion and thus may retard or prevent the development of experimental atherosclerosis even in the presence of marked and sustained hypercholesterolemia.

### *Discussion*

(Dr. Paul Kimmelstiel, Charlotte, N.C.) I was very much interested in Dr. Kellner's experiments, and I would like to make an additional comment. Not only phospholipids, but also other lipids, including cerebrosides, are involved in the atherosclerotic process. Approximately 19 years ago I conducted experiments along the same line, producing atherosclerosis in rabbits by feeding them pure cholesterol. On examining the organs of these rabbits it was found that not only cholesterol, but also phospholipids and cerebrosides, increased in amount parallel to the extent of atherosclerosis. This fact substantiates the assumption that not only phospholipids, but also cerebrosides, aid in the colloidal suspension of cholesterol. At the time I thought that an increase of phospholipids and cerebrosides, both emulsifying cholesterol, may prevent experimental atherosclerosis.

(Dr. Russell L. Holman, New Orleans, La.) I would like to ask Dr. Kellner if he has ideas concerning any possible detergent agent which might be used in man, or any metabolic process that might occur in man that might increase the plasma phospholipid levels.

(Dr. G. Lyman Duff, Montreal, Que.) I think Dr. Kellner's work is very interesting, and his results fit in very well with some experiments we have conducted lately. About 2 years ago Dr. McMillan and I were able to show in a quite different experiment a similar inhibitory effect which we did not understand at the outset. Cholesterol, in the same quantities and for the same period, was fed to a control group of rabbits and to another group in which there had already been established a severe alloxan diabetes. In the diabetic group, quite contrary to expectation, there was a marked, and in most cases, a complete inhibition of the development of atherosclerosis, even though the blood cholesterol level had risen to practically the same degree in both groups; in fact, if there was any difference between the two groups, the cholesterol levels rose higher in the diabetic than in the non-diabetic animals, and yet the former were those in which the process of atherosclerosis was inhibited. Later study of the plasma lipids with Dr. Torrence Payne showed that there was a great difference in the plasma lipid constituents other than cholesterol, although the changes in the cholesterol levels were practically the same in the two

groups of animals. The phospholipids in the control group rose, as Dr. Kellner has shown, only moderately, and neutral fats practically not at all. On the contrary, in the diabetic animals that were protected against atherosclerosis, the phospholipids rose quite markedly, but in addition the neutral fats rose even more. For this reason, I wonder whether the solubility and stability of cholesterol in the plasma should not be considered as a function of all the plasma lipids, not merely of the phospholipids alone.

Dr. Kellner also spoke of the importance of the association of cholesterol with plasma proteins in lipoprotein complexes. We made determinations by a method which may not be reliable (and all methods are apparently subject to question) of the proportions of all the plasma lipid fractions firmly attached to proteins as compared with the proportions that are free, or only loosely bound to proteins. In the two groups of animals already mentioned there was no difference in the proportions of the three lipid fractions firmly attached to proteins. The greater part of the increase in blood lipids was accounted for by lipids not firmly bound to protein, but in this respect the control and the diabetic groups were the same. Judging from these results, which I admit are unreliable because we are not sure the method is reliable, it would appear that the attachment of cholesterol to protein is not so important in maintaining its stability as is its relation to the other lipids in the plasma.

(Dr. Kellner) With regard to the cerebrosides playing a rôle in the emulsification of the serum lipids, they may play such a rôle, but I think it is a minor one, because in mammals it has been shown that the bulk of the phospholipids in the serum are lecithins.

The surface-active agents used in these experiments are far too toxic for clinical use, and I do not know of any detergent at present that can be injected with safety into man.

We have observed results similar to those described by Dr. Duff in acute alloxan diabetes. In rabbits injected with alloxan and maintained on a normal diet there quickly developed a lipemia, with a marked elevation of both cholesterol and phospholipid, usually to about the same degree. In the alloxan-diabetic animals fed cholesterol the increase in phospholipids was not as great as the increase in cholesterol. The neutral fat may play a rôle in the emulsification of cholesterol, but as between phospholipids and neutral fat, I think the phospholipids are far better emulsifying agents.

As far as lipoproteins are concerned, I cannot agree with Dr. Duff. I think the evidence indicates that all the lipids in the serum are attached to protein. The studies on protein fractionation from Cohn's laboratory in Boston have shown that there is little or no serum lipid which is not attached to protein. I feel the lipoproteins are extremely important, probably more important than phospholipid in maintaining the stability of the serum lipid emulsion.

THE PATHOGENESIS OF AMNIOTIC FLUID EMBOLISM. Benjamin H. Landing and (by invitation) Olga Leary, Jr., Boston, Mass.

*Abstract.* The results of study of series of placentas from cases of premature non-toxic marginal separation; of the uteri and lungs from cases of hysterectomy or death following post-partum hemorrhage, cesarean section, or ruptured uterus; and of control series of post-partum uteri and lungs of cases of maternal death in the first post-partum week, are reported from the point of view of evidences of entrance of amniotic fluid into the maternal circulation. Amniotic fluid appears to enter the maternal circulation only by way of uterine or placental vessels opened abnormally, as in cases of placenta accreta, cesarean section, ruptured uterus, partial retention of the placenta, and probably also premature marginal separation of the placenta. Evidences of such entrance were found more frequently than clinical

amniotic fluid embolism, so that non-fatal and sub-clinical cases are probably more common than is realized. In no case were evidences of entrance of amniotic fluid into the vessels of a normal placental site observed.

A Comparison of Cutaneous and Pulmonary Lesions in Experimental Silicosis. R. B. Anderson (by invitation) and F. D. Gunn, Salt Lake City, Utah.

*Abstract.* Previous experience with various routes of injection of siliceous dusts in laboratory animals has failed to discover a method which yields sufficiently consistent results to justify statistical comparisons between the desmoplastic effects of selected samples of dusts. Intraperitoneal or intravenous injections of mice, intraperitoneal injections of guinea-pigs and intradermal or subcutaneous injections of rabbits have been found to have serious disadvantages for one reason or another. Of these methods the subcutaneous injection of rabbits has, in our hands, yielded the most consistent results. The dust chamber method requires expensive equipment and long periods of time running into years.

In an attempt to devise a more accurate method for the rapid production of silicotic lesions we have tested the effects of silica upon the parenchyma of the rat's lung after intrabronchial insufflation. Adult white male rats were subjected under ether anesthesia to intrabronchial insufflation of measured quantities of pure silica and siliceous dusts from mines, suspended in gastric mucin. Purified gastric mucin (5 per cent) dissolved) in physiologic saline solution is practically non-irritating to the pulmonary parenchyma and serves to retain the suspended dust in the lung until phagocytosis has occurred. The samples of dust were elutriated so that the particle size was less than 5 $\mu$ and injected in 10 mg. doses, suspended in 0.1 cc. of mucin. Pulverized quartz and finely ground blood charcoal (C.P.) were similarly elutriated and used as control samples. Samples of lesions were examined routinely in paraffin sections, stained for connective tissue, and selected samples were examined in micro-incineration preparations for the presence and distribution of silica. Rats from each group were killed and examined at 1, 3, 6, and 9 months. Fibrosis of the silica-bearing tissue was progressive throughout this period, and comparative values could be assigned as early as 3 months. In the pure carbon control, no evidence of fibrosis was detectable in the lungs. Finely divided pure silica in 10 mg. doses elicited a strong desmoplastic reaction associated with greatly increased macrophages as early as 1 month after injection. In the lungs necrosis was minimal or absent and dense fibrous nodules were present at 1 month, the earliest period at which animals were sacrificed. The nodules were histologically similar to those in nodular silicosis of the human lung.

For the purposes of comparison, rabbits were injected intradermally with 5.0 mg. doses in distilled water, or subcutaneously with 50 mg. doses, and sacrificed at intervals of 1, 2, 4, 6, and 12 months. When the results of similar doses were compared, fibrosis in the skin of rabbits comparable in degree with that seen in rats' lungs required a much longer time and assumed the form of a fibrous capsule around a central mass of macrophages and necrotic tissue. Necrosis occurred frequently in the skin and subcutaneous tissue, since the injected silica was concentrated in relatively small masses of tissue.

*Discussion*

(Dr. Paul Gross, Pittsburgh, Pa.) Dr. Brown and I, in our study of pneumoconiosis at the Industrial Hygiene Foundation, have been using a somewhat similar method not only with silica, but also iron oxide, carbon, and other materials. We carefully controlled the particle size, which was generally less than 1 $\mu$ in diameter. We used albino rats and injected suspensions of the material into the trachea, without making any skin incision. We were able to locate our puncture and do these intratracheal insufflations of various dusts at a fairly high rate of speed. We have

been able to produce fibrosis in the lungs in as little as 3 weeks. We have also injected carbon particles 0.2 $\mu$ or less in size, although by the hematoxylin and eosin stain we were unable to demonstrate any significant amount of fibrosis. However, by means of the reticulum stain a well defined reticulinosis was demonstrated. An even more marked reticulinosis is demonstrable in our sections in about 6 weeks with finely divided iron oxide.

(Dr. Anderson) Our technic has been to anesthetize rats and insufflate the mucinous suspension of dust by way of a ureteral catheter inserted through the bronchoscope. By this method it is possible to place all of the insufflate into the left lung. This made accurate comparisons of the lesions possible, which was our main purpose. We did not examine animals at less than 1 month, but at 1 month pure silica had resulted in the formation of fibrous nodules.

THE DEMONSTRATION OF AN EFFECT OF PARATHYROID EXTRACT ON BONE MATRIX. William H. Carnes, Baltimore, Md.

*Abstract.* Theories of the mechanism of action of parathyroid hormone have been dominated by the idea that the hormone modifies an equilibrium between the solid phase of bone mineral and the ionic calcium and phosphorus in the blood and tissue fluids. Direct observations on the bones of parathyroid-treated animals, however, have failed to show evidence of the removal of mineral prior to the destruction of the matrix, which is implied by this concept. Moreover, unpublished experiments have shown no initial decrease in the ratio, ash/matrix, of such bones. There is an alternative hypothesis that the mobilization of mineral is a by-product of the primary destruction of bone. Such a notion would be supported by the demonstration that parathyroid hormone has an effect on non-calcified bone matrix (osteoid) similar to its effect on whole bone. Hooded male rats were weaned at 3 to 4 weeks of age on a purified diet of low calcium and phosphorus content. After 4 weeks on the diet, parathyroid extract (Lilly) was administered intraperitoneally in doses of 125 U.S.P. units twice daily for various periods from 8 to 72 hours. Ribs and long bones were fixed in 10 per cent neutral formalin, embedded in paraffin, cut without decalcification, and examined by the von Kossa method. The development of osteitis fibrosa in the rachitic rats was quite comparable to that in similarly treated non-rachitic rats on the same basal diet containing ample calcium and phosphorus. Resorption of osteoid, as judged by the appearance of lacunae containing osteoclasts, had begun within 8 hours. There was progressive resorption of the rachitic metaphysis at later intervals accompanied by the re-appearance of a provisional zone of calcification in the cartilage, re-establishment of orderly proliferation and removal of cartilage cells, and calcification of remaining chondro-osteoid matrix. By 72 hours the diaphyseal osteoid had also completely calcified. Paul Rubin has observed essentially identical sequences in the callus of fractured fibulas in some of these animals. The serum calcium rose to a maximum of 12.2 mg. per cent in 72 hours, whereas the serum phosphorus showed no consistent change and there was no metastatic calcification in the kidneys. In contrast, treated rats on a diet containing ample calcium and phosphorus had a higher serum calcium, an initial fall in serum phosphorus followed by a rise, and developed extensive renal calcification. These observations indicate that (1) bone matrix may be destroyed by the action of parathyroid hormone irrespective of its mineral content, (2) the levels of serum calcium and metastatic calcification are a function of the mineral content of the resorbed matrix, and (3) calcification may be induced in cartilage and osteoid matrix through its action. These facts are not consistent with the theory that the hormone acts primarily upon some mechanism that causes solution of bone mineral but require the conclusion that its action on bone is mediated through an effect primarily upon the organic matrix.

THE CELL TYPE OF SECONDARY PARATHYROID HYPERPLASIA.* Osborne A. Brines and (by invitation) George E. Fritz, Detroit, Mich.

*Abstract.* It has been well established that secondary parathyroid hyperplasia may result from renal disease. The normal-sized chief cell has been thought to be the predominant cell in the majority of cases of parathyroid hyperplasia secondary to renal disease, and since 1937 the parathyroid enlargement in these cases has been called "chief cell hyperplasia." This has been contrasted with "wasserhelle" cell or "primary" hyperplasia. All authors, however, do not agree that the normal-sized chief cell is usually predominant in parathyroid enlargement secondary to renal disease.

The purpose of this paper is to report the findings of a study of the parathyroid gland in 61 cases of bilateral renal disease with particular emphasis on cell type. For control material the parathyroid glands from 76 cases without renal disease were studied and compared. A definite increase in weight of the parathyroid glands was found in the cases of renal disease. The increase in weight was proportionate to the severity and duration of the renal damage. The predominant parathyroid cell in the majority of cases studied was the vesicular cell. The size of the cells was not constant, but in many cases the cells were of the large typical "wasserhelle" type. In the majority of cases of mild renal disease of short duration the parathyroid glands contained approximately equal numbers of chief and vesicular cells. Transitions between the cell types could be seen. In no case of severe renal disease of long standing was the normal-sized chief cell predominant.

These findings indicate that the vesicular cell is usually the predominant parathyroid cell type in parathyroid hyperplasia secondary to renal disease.

### *Discussion*

(Dr. B. Earl Clarke, New York, N.Y.). I would like to ask the age of the patients in your series. I understand that normally in children up to 10 or 12 years of age there are nothing but chief cells. In one of the cases I reported this morning there was definite enlargement of the parathyroid glands which I am sure was secondary to the marked renal damage, and there were only chief cells.

(Dr. Benjamin Castleman, Boston, Mass.) I should like to ask first whether the size of the nucleus in these clear cells was the same as that in the chief cells, and second, how the tissue was prepared. We have found that quite often in formalin fixation there is enlargement of the cell with the so-called water-clear structure. It is true that in secondary hyperplasia one often finds small foci of clear cells, but it is as a rule not a common finding. We have seen it in cases of true rickets and in one case of renal failure associated with hypercalcemia without hypercalcuria or hypophosphatemia. There is quite a difference, however, between the clear cell of the normal or secondarily hyperplastic gland, which is just a modification of the ordinary chief cell, and the cell found in so-called primary hyperplasia, which is a very much larger cell.

(Dr. F. D. Gunn, Salt Lake City, Utah) I should like to ask whether studies were made on the glycogen content of the cells in alcohol-fixed tissue.

(Dr. Tracy B. Mallory, Boston, Mass.) I think it might be pertinent to ask Dr. Fritz if he can define a little more exactly what he considers the difference between the chief cell and the water-clear cell. As he has said and shown in his slides, one sees all grades of transition from one to the other, and I think it is very difficult to draw a sharp dividing line. It seems very probable to me, as Dr. Castleman has pointed out, that the dividing line might be drawn quite differently when different fixatives and different embedding technics are used.

* This article will appear in a subsequent issue of *The American Journal of Pathology*.

(Dr. Fritz) In answer to Dr. Clarke's question, all cases in this series were adults, with the exception of one child aged 14. It was my impression that the only parathyroid cell type not present in children is the oxyphil cell.

In reply to Dr. Castleman, we believe that the nuclei of the clear cells are identical. All of the tissue was formalin-fixed, as is our routine autopsy material. We have studied no case of primary hyperplasia, so have been unable to compare the nuclei of these cells with those of primary hyperplasia. Some of the cells in this series measure up to 40 $\mu$.

In reply to Dr. Gunn's question regarding glycogen content, glycogen stains were not prepared. Glycogen studies were made by Castleman and Mallory who found an increased glycogen content in mild renal disease.

In reply to Dr. Mallory's question regarding the definition of cells, the following criteria were used: The chief cell has solid cytoplasm, the vesicular cell has a vacuolated cytoplasm, and the clear cell has a clear cytoplasm. The nuclei in all three types were identical. It is our opinion that the clear cell is a transition stage of the chief cell.

CHANGES IN THE PANCREATIC ISLETS OF LANGERHANS IN ADDISON'S DISEASE. Dorin L. Hinerman (by invitation), Ann Arbor, Mich.

*Abstract.* Having in mind the effects of the adrenal cortical hormones upon carbohydrate metabolism, the islets of Langerhans were studied in 18 cases of Addison's disease. In this disease there is a profound reduction in the insulin requirement and the patients exhibit a marked sensitivity to insulin even when diabetes mellitus is co-existent. Therefore the finding of a marked degree of hyperplasia of the islets of Langerhans in every case of Addison's disease is a surprising feature in view of the reduced functional requirements, and all the more striking in view of the atrophy of all other organs, which is a common feature of Addison's disease. The criteria for hyperplasia of the islets are increased numbers of islets (making allowance for acinar atrophy), increased size of islets due to increased number of cells, and an active genesis of new islets from proliferating ducts and ductules. In some cases the combination of acinar atrophy and proliferating ductules results in a picture resembling fetal and infantile neogenesis of islets of Langerhans.

In determining the nature of the cell types involved in the hyperplastic process, Gomori's chromium hematoxylin and phloxine stain differentiates beta cells from the alpha cells and delta cells of Bloom. Mallory's Heidenhain-azan stain and Mallory's phosphotungstic acid-hematoxylin method differentiate alpha cells from delta cells of Bloom. The Gomori method is the most useful granule stain. In normal islets the beta cell is the predominate cell type. These cells possess fine dust-like blue cytoplasmic granules which are precursors of insulin. Alpha and delta cells have a pink to red cytoplasm. The alpha cells are less numerous than beta cells. Recently a glycogenolytic hormone has been attributed to them. Regardless of this theory, they are probably immature beta cells. The infrequent delta cells of Bloom are probably degenerative forms of the other islet cells.

In Addison's disease all cell types are increased with the greatest increase in alpha cells. Therefore the average percentage ratio of beta to alpha cells is decreased from about 80%/30% to about 50%/50%. Therefore the beta-alpha ratio approaches that of diabetes mellitus. However in Addison's disease there is an increase in the total number of apparently normal beta cells. In addition, the beta cells possess unusually abundant granules suggesting retention of insulin because of a lack of peripheral demand. Actually the changes in islets of Langerhans in Addison's disease are similar to but more pronounced than those in most examples of active islet hyperplasia. Hyperplasia of islets is most often compensatory to islet cell destruction. In the cases of Addison's disease there were no apparent destructive lesions in the pancreas with the possible exception of amyloidosis in 2 of the 18 cases.

Another possible explanation for hyperplasia can be eliminated because the functional demand on islet cells is reduced in Addison's disease. Therefore the total hyperplasia of islet cells is probably due to the removal of an inhibitory influence which is normally exerted directly upon the islets of Langerhans in the presence of the adrenal cortical hormones. When this inhibitory factor is removed in Addison's disease, hyperplasia results.

*Discussion*

(Dr. Robert A. Moore, St. Louis, Mo.) Is there any difference in the incidence of this lesion in relation to the cause of the Addison's disease? I have in mind the changes in the thyroid gland which are more frequently associated with primary atrophy than with tuberculosis.

(Dr. Hinerman) These cases represent all of the common types of etiologic agents for Addison's disease: tuberculosis, histoplasmosis in one case, amyloidosis, and primary atrophy. In all cases there was practically the same degree of hyperplasia; I could discover no difference.

GLYCOGEN INFILTRATION (SO-CALLED "HYDROPIC DEGENERATION") OF THE PANCREAS IN EXPERIMENTAL AND HUMAN DIABETES MELLITUS. Wilfred E. Toreson (by invitation), Montreal, Que.

*Abstract.* All authors are agreed that the cytoplasmic vacuoles of pancreatic islet and ductule cells affected by "hydropic degeneration" are devoid of any demonstrable content; hence, it is presumed, such vacuoles contain only excessive amounts of water. However, large quantities of glycogen are readily demonstrable in the "hydropic" cells of the pancreas in experimental diabetes. Helly-fixed pancreas from rabbits made permanently diabetic with alloxan is especially suitable material. Chemical fixation displaces glycogen toward the periphery of most but not all affected cells. However, the combination of fixation by freezing, dehydration in vacuo and direct embedding in paraffin leaves glycogen evenly dispersed throughout the cytoplasm. Chemical analysis of the pancreas of the alloxan diabetic rabbit in which severe "hydropic degeneration" is present may yield as much as 0.0025 gm. of glycogen per gm. of tissue (0.250 per cent).

Studies of the pancreases from cases of diabetes mellitus in which death occurred in coma were made. Both the periodic acid-Schiff's reagent reaction and the Best carmine method were utilized following application of a celloidin film to the mounted sections. Most of the material was obtained from autopsies performed between the years 1926 and 1933. It had been fixed in formalin and embedded in paraffin. When stained by ordinary methods "hydropic degeneration" was apparent in 6 of 12 pancreases. Glycogen was demonstrable in the distended, vacuolated islet cells in 4 of the 6. In one, Bouin-fixed material showed numerous conspicuous, large spherical granules of glycogen; in the others, in which only formalin-fixed material was available, lesser quantities were identified. In one of the cases in which glycogen could not be demonstrated in the vacuolated cells, insulin therapy had lowered the blood sugar level to 70 mg. per 100 cc. and autopsy had been delayed for 22 hours following death. In the other, extensive severe hyalinization also affected the islets, so that the vacuolation might have represented fatty change.

The abnormal content of glycogen in the cytoplasm of cells showing so-called "hydropic degeneration" is more responsible for the characteristic vacuolation and swelling apparent in ordinary histologic preparations than is the presence of excessive water. The latter is probably a consequence of the accumulation of glycogen. This lesion of the pancreatic islet and ductule cells would be more precisely designated "glycogen infiltration." It is the histopathologic common denominator of human and experimental diabetes mellitus. Confirmation of the suspected existence of this unique lesion in human pancreases may be attained by utilizing appropriate methods of fixation and staining for the demonstration of glycogen.

RECENT ADVANCES IN MICROSCOPY: THE REFLECTING MICROSCOPE. Robert C. Mellors (by invitation) and C. P. Rhoads, New York, N.Y.

*Abstract.* In this communication it is shown how recent developments in the optics of the reflecting microscope make it possible to undertake a study of the chemistry of intact cells. Among the potentially useful technics is a spectroscopic method which permits the recording of the ultraviolet absorption characteristics of cells within a period of a few seconds. The apparatus employed consists of a light source which emits radiation throughout the ultraviolet and the visible spectrum, a microscope with a reflecting condenser and objective, and a quartz spectograph. This analytic system permits the simultaneous recording of a series of discrete, or overlapping, monochromatic, cellular images of which there exists one for each wave length of light emitted by the source.

Preliminary application of this method has been made to the study of exfoliated cells, bone marrow smears, ultra-thin tissue sections, and other material of interest in pathology. In appropriate cells it has been possible to detect in the visible and the ultraviolet spectrum, light absorption which is presumably due to the presence of such compounds as the porphyrins, cyclic amino acids, purine and pyrimidine polynucleotides, steroids, etc.

An analytic system for the study of living cells consists of a monochromator (wave length selector), reflecting microscope, and the appropriate photographic accessories. The ultraviolet light absorption of nuclear material is low and remarkably similar for a variety of living, resting cells, whereas during mitotic division or after injury by physical or chemical agents, the absorption is greatly increased.

## READ BY TITLE

THE "LUPUS ERYTHEMATOSUS PHENOMENA" OF BLOOD AND BONE MARROW. MORPHOLOGIC AND SEROLOGIC OBSERVATIONS. Lawrence Berman and (by invitation) Arnold R. Axelrod, Herbert L. Goodman, and Robert I. McClaughry, Detroit, Mich.

*Abstract.* Recent studies of blood and bone marrow provide useful adjuncts to the diagnosis of acute disseminated lupus erythematosus. The characteristic phenomena include (1) degeneration of nuclei of leukocytes, (2) phagocytosis of the altered masses of nuclear origin, (3) the appearance of clusters of neutrophils about masses of debris. Special methods of fixation and staining of marrow smears, devised by the authors, yield convenient material for rapid scanning of smears for diagnostic purposes.

A quantitative study of the incidence of the various LE (lupus erythematosus) phenomena indicates that the phenomena are not unique qualitative findings, as they have been observed in patients free of the disease. The practical diagnostic importance of the changes is discussed in the light of quantitative studies. The authors' observations support the concept that the LE cell inclusions seen in marrow films are identical with the "hematoxylin-staining bodies" (Klemperer *et al.*) seen in tissues of patients with the disease. Histochemical studies failed to substantiate the theory that the changes in LE marrows are related to depolymerization of desoxyribose nucleic acid (Klemperer *et al.*). Investigation of the *in vitro* test for lupus erythematosus, in which plasma from LE patients is placed in contact with marrow material from normal persons or other patients, yields the following facts:

1. LE plasma contains a factor which promotes or enhances phagocytosis of nuclear masses by leukocytes from normal blood.

2. Bone marrow material from patients with various diseases reacts with varying degrees to contact with plasma from LE patients. Cells from certain patients fail to react.

3. Splenectomy had no effect on the incidence of the LE phenomena in the

marrow smears of a patient with the disease, nor on the activity of the plasma from a patient with the disease, as observed in the *in vitro* test.

4. LE plasma can be stored in the deep freezer for over 4 months without loss of activity for the *in vitro* reaction.

5. Post-mortem serum from a patient with acute disseminated lupus erythematosus gave positive diagnostic results in the *in vitro* test.

6. Cells of animal origin can be used for the *in vitro* test.

7. The activity of LE plasma is augmented after having been used once for the *in vitro* test.

8. Activity of LE plasma resides in a factor associated with the gamma globulin fraction.

VISUALIZATION OF DEHYDROGENASE ACTIVITY IN TISSUES. Maurice M. Black, Francis D. Speer (by invitation), and S. R. Opler, New York, N.Y.

*Abstract.* The reduction of tetrazolium salts by tissue slices appears to reflect the dehydrogenase activity of tissues. Marked differences in the degree of reduction of the tetrazolium were found among different organs, and within any organ type further differences were observed among the tissue components of the organ. Cytologic observations of scrapings from normal and malignant tissues indicate that the formazan is localized exclusively in the cytoplasm while the nuclei remain colorless.

The organs and tissues studied may be divided into two groups on the basis of their dehydrogenase activity, *viz.*, those with a high degree of activity as evidenced by the production of the red formazan and those with a lower or minimal dehydrogenase activity manifested by an absence or minimal amount of color produced. Most epithelial structures possess a high degree of dehydrogenase activity. When malignant tumors arise from such tissues, this activity is maintained in the malignant growth, *viz.*, carcinoma of the breast, stomach, rectum, etc., and such activity persists in the local invasive process as well as in the metastatic lesions. Active reduction of the tetrazolium occurs also in the enlarged lymph nodes of acute myeloblastic leukemia and reticulum cell lymphosarcoma, while normal nodes and those from cases of Hodgkin's disease and chronic lymphatic leukemia fail to reduce the dye appreciably. Tissue slices from fibrosarcoma also reduce the activity in contrast to the minimal activity found in normal fibrous connective tissue and inflammatory granulomata.

In normal breast sections, the fibrous tissue stroma appears as a white background throughout which are scattered punctate areas of red, indicating the location of the ducts. With the advent of cystic or adenomatous changes, these punctate areas may be more numerous or larger in size, but in no case do they lose the distinct separation from the unstained stroma and fat. In cases of breast carcinoma the distinct architectural integrity is replaced by solid red staining areas, the outlines and intensity of which reflect the invasion of the stroma and replacement of parenchyma by the malignant cells. This alteration in the normal architectural patterns of the tissue components of an organ in the presence of cancer is evident also in other areas than the breast. In all cases studied the invading lesion was distinctly delineated by the diffuse red color produced by the tumor cells in areas that should normally have minimal color after incubation with the tetrazolium solution. This phenomenon has proved useful as an aid in frozen section tissue examination where a rapid decision is needed as to the malignancy or benignancy of a growth. In addition it provides excellent visualization of metastatic spread to lymph nodes, particularly where such nodes are only partially involved.

INCIDENCE OF SUBCLINICAL POLIOMYELITIS IN AN URBAN AREA, ACCORDING TO AGE GROUPS. Albert E. Casey and (by invitation) William I. Fishbein, F. M. Schabel, Jr., and H. T. Smith, Chicago, Ill.

*Abstract.* Stools were collected in Chicago during 1945 and 1946 from 260 contact, non-contact, and control children. From the stools a total of 576 monkeys were

inoculated. Virus was recovered from the stools of 54 of 101 contact, 6 of 55 non-contact, and 8 of 104 control children. Twelve (86 per cent) of the 14 positive non-contact and control children were 1 to 4 years of age. Eight (80 per cent) of 10 familial contacts and 38 (78 per cent) of 49 non-familial contacts, 1 to 5 years of age, were positive; 6 (75 per cent) of 8 familial contacts, and 7 (23 per cent) among 30 non-familial contacts, 6 years of age and older, were positive. It was calculated that most children in Chicago probably had had poliomyelitis once by the fourth birthday, 75 per cent perhaps twice by the sixth birthday, either different strains or re-infection. From these calculations it would appear that on the average over 100,000 to 125,000 cases of poliomyelitis occur in Chicago each year, or 3 to 4 per cent of the total population. There is a similarity in the estimates of poliomyelitis infection in urban populations in relation to age, whether determined by virus isolation, by sparing after virus challenge, or by neutralizing antibody response.

ANOMALIES OF THE CORONARY ARTERIES. REPORT OF TWO CASES, WITH A COMMENT ON THE DYNAMICS OF DEVELOPMENT OF THE CORONARY CIRCULATION. Frank R. Dutra, Cincinnati, Ohio.

*Abstract.* The effects of congenital origin of the left coronary artery from the right pulmonary artery are contrasted with congenital absence of the left coronary artery. The wall of the left ventricle in the former was hypertrophied and there were diffuse and focal fibrosis and multiple infarcts. There was no evidence of a tendency toward hyperplasia of the right coronary artery to compensate for the hypoxia of the myocardium of the left ventricle. With complete absence of the left coronary artery, branches of the right coronary artery extended into the wall of the left ventricle and supplied sufficient blood so that there was no alteration of the myocardium. In the several cases of abnormal origin of the left coronary artery from the pulmonary artery which have been reported previously, the persons have nearly all died in infancy, and with fibrosis and infarcts of the myocardium of the left ventricle; persons with congenital absence of the left coronary artery have had no other cardiac lesions and did not develop symptoms nor die as a result of the anomaly. The development of supplementary arterial channels, which compensate for an inadequate supply of blood to the myocardium of the left ventricle in the absence of the left coronary artery, appears to depend upon the absence of vessels carrying blood under arterial pressure in that area of the myocardium. The presence of blood under arterial pressure in the wall of the left ventricle is sufficient to prevent the development of supplementary vessels from the right coronary artery, even though the blood being distributed in the left coronary artery includes blood which has an oxygen-tension like that of venous blood.

METASTASIZING CARCINOMA OF THE PARATHYROID WITH OSTEITIS FIBROSA CYSTICA AND EXTENSIVE CALCINOSIS. John T. Ellis (by invitation) and Sung Soo Lee (by invitation), New York, N.Y.

*Abstract.* This case provides one of the few examples of metastasizing carcinoma of the parathyroid examined post mortem, and it has unusual interest because of the functional derangements produced by the tumor. At death the patient was 29 years of age, symptoms of hyperparathyroidism having been present intermittently for 6 years. These began with the first pregnancy at the age of 23. Approximately 1 year later a cystic lesion of the mandible was diagnosed by biopsy as giant cell tumor and osteitis fibrosa. Six months later generalized osteitis fibrosa cystica and hypercalcemia were demonstrated and a solitary parathyroid adenoma outside the thyroid gland was removed. This resulted in complete remission of the disease for 2½ years followed by an exacerbation with extensive diffuse calcification of the kidneys during the third pregnancy. Two parathyroid adenomas measuring 1.5 and 1.2 cm. respectively were removed from the left. Three months later the left lobe of the thyroid gland was removed; within it there were two white, firm, encapsulated nodules, 1.5

cm. in diameter, which were diagnosed as carcinoma. Because of continued symptoms and hypercalcemia, two metastatic nodules measuring 0.9 and 1.2 cm. were found and removed from the upper and lower lobes of the left lung. Manifestations of the disease continued, and 4 months later blanching, pain, and coldness of the extremities developed. These were thought to be due to arterial calcification and insufficiency. Death occurred a short time later.

Post-mortem examination revealed a 1.0 cm. local recurrence of the tumor in the left sternocleidomastoid muscle, a 1.0 cm. nodule in a left supraclavicular lymph node, and a 4 by 5 by 5 cm., gray, firm, solitary metastasis in the left lobe of the liver. Microscopically, tumor from all of these sites was composed of sheets of chief cells which formed small cystic spaces in some areas. Three small parathyroid glands removed from the posterior surface of the right lobe of the thyroid gland were partially replaced by dense fibrous tissue. X-ray examination of the ribs, vertebrae, calvarium, and femur revealed osteoporosis and cyst formation, and microscopically there was active resorption of lamellar bone associated with new bone formation and fibrous tissue replacement of the marrow. Massive calcium deposition was demonstrated in the media of medium and small-sized arteries. In the right lung there were numerous 4 to 6 cm. areas of almost solid calcification in regions distal to thrombosed pulmonary arteries. The remainder of the lung showed moderate calcinosis. The medullary portions of the kidney contained large concentric masses of calcium salts, while in the cortex calcium was found in the basement membrane of the tubules, within tubules, in Bowman's membrane, and in the walls of the glomerular capillaries. Calcium was present also in the papillary muscles of the left ventricle of the heart at the point of insertion of the chordae tendineae. Isolated muscle fibers of the heart as well as many small arteries were calcified. Much calcium was contained within dilated gastric glands and in interstitial tissue. Smaller pancreatic ducts were dilated and filled with partially calcified débris, while the parenchyma was atrophic, fibrosed, and infiltrated with lymphocytes.

GIANT CELL FOREIGN BODY REACTION TO NON-CHOLESTEROL LIPID PLATE CRYSTALS. A CASE REPORT WITH CHEMICAL STUDIES. Ralph L. Engle, Jr. (by invitation), New York. N.Y.

*Abstract.* A 68-year-old white maid developed asthma and 1 year later died in respiratory distress during bronchoscopic examination under local procaine anesthesia. At autopsy the upper and middle lobes of the right lung were markedly fibrosed. On microscopic examination of paraffin-embedded and stained preparations a giant cell foreign body reaction was found with plate crystal slits and asteroid bodies in the giant cells. The plate crystals themselves were seen in frozen sections of tissue not exposed to the usual fat solvents. With the dissecting microscope these crystals were separated from the tissue. They proved to be octagonal and anisotropic, with a melting point of 77° to 78°C. (microscopic technic). They were soluble at room temperature in ether, chloroform, carbon tetrachloride, benzene, hexane, xylol, hot ethanol, and acetone; they proved insoluble in water, cold ethanol, acetone, methanol, N/10 hydrochloric acid, N/10 sodium hydroxide, sodium bicarbonate solution, dilute and glacial acetic acid, and ethyl acetate. Solutions of the washed crystals gave a positive Liebermann-Burchardt reaction. In other experiments the crystals were extracted from the tissue by means of organic solvents. The extracted material, both before and after hydrolysis, gave no indication of cholesterol when submitted to analysis by means of infra-red absorption. Considered together the findings make it evident that the crystals were a steroid other than cholesterol or an ester of cholesterol.

The lipid dissolved in acetone-ether crystallized out slowly as octagonal plates, but when dissolved in ether alone it crystallized out rapidly, exhibiting asteroid forms. These were similar in shape to the asteroid bodies present in the foreign body giant cells. In the latter, however, the asteroids were not refractile, even in

preparations unexposed to fat solvents, hence they presumably were not crystalline. On the contrary, the asteroid spicules in giant cells had the appearance of condensed cytoplasm in stained and unstained preparations as well, though not infrequently they lay in close proximity to the lipid plate crystals within the same cytoplasmic masses.

SPONGE BIOPSY IN THE DIAGNOSIS OF RECTAL AND SIGMOIDAL CANCER. Sidney A. Gladstone, New York, N.Y.

*Abstract.* The diagnosis of cancer in ulcerating lesions of the rectum and sigmoid may be made by sponge biopsy. This method depends on the microscopic examination of small tissue particles absorbed in the pores of a suitable sponge which has been firmly rubbed over the base and margins of the suspected ulcer. In a series of 32 patients, sponge biopsy was positive for cancer in 15 cases. In 14 of these the diagnosis was confirmed by the examination of additional tissue obtained by surgical biopsy or operative surgical specimen. In one case, missed by surgical biopsy, the sponge biopsy showed an abundance of characteristic cancerous tissue. At operation this patient presented an annular lesion of the colon with metastases to the liver. Among the 15 cancer cases, a second instance occurred in which surgical biopsy missed the cancer, which was adequately diagnosed by sponge biopsy. A *single* piece of tissue removed by surgical biopsy may miss the cancer. Such failure is less likely to occur during sponge biopsy because this method provides for the procurement of *many* small tissue particles from different areas of the ulcer base and margins.

The advantages of sponge biopsy in the diagnosis of cancer in ulcerating lesions of the rectum and sigmoid are several. The method is easily applied. The patient suffers no pain. The danger of causing hemorrhage or deep infection is negligible in comparison with surgical biopsy. With respect to accurary and reliability the method of sponge biopsy closely approximates the method of surgical biopsy.

THE RÔLE OF DEGENERATION IN ABNORMAL DEVELOPMENT. Peter Gruenwald (by invitation), Brooklyn, N.Y.

*Abstract.* It had been assumed for many years that most malformations are the result of deviations or arrest of normal developmental processes, and on this basis many investigators figured out how and at which stage a malformation known only in its final form should have developed. More recently, however, degeneration of previously well formed parts has been recognized as one of the fundamental mechanisms of maldevelopment. This has been found to occur in embryonic stages of hereditary malformations of the brain, eyes, ears, feet, tail, and other parts of laboratory animals. A pertinent example is brachydactyly in the rabbit (Greene and Saxton, *J. Exper. Med.*, 1939, 69, 301–314). The feet appear normal until the 18th day of gestation, when hemorrhage and necrosis set in, followed by sloughing.

Several lines of our own research point to the significance of similar processes: In a particular form of hereditary microphthalmia in chicks the amount of retinal tissue is at first normal; then rosettes and folds form and much of their tissue later becomes necrotic, thus leaving at the end of incubation a greatly reduced eye. The development of malformations produced in chick embryos by selenium compounds is being examined in this laboratory. Even though the agent is introduced into the egg before the onset of incubation, development of the nervous system proceeds normally until the fourth day, when extensive necrosis occurs in a characteristic pattern of distribution throughout brain and spinal cord. Malformations arise in the previously well formed extremities at even later stages.

In sporadic malformations in man developmental stages are usually not available for study; yet, secondary degeneration must be postulated in some instances; and probably occurs in others as well. Atresias of the intestine in an otherwise well formed body cannot be the result of primary failure of development, for the fetal

body cannot form unless a tubular gut is formed at the same time. Actually they must develop late, at least in some instances, because we have found ingested cornified cells embedded in granulation tissue at the site of the atresia, or even distal to it. Most of the so-called amniotic amputations and other defects allegedly caused by amniotic bands are, according to recent concepts, primarily due to inherent defective potentialities of the tissues leading to ulceration and changes similar to those described above for the rabbit; adhesions of the amnion are secondary. There is an obvious similarity of these changes with hereditary or "idiopathic" degenerative diseases occurring in man after birth, often at an advanced age. In fact, some of the above-mentioned hereditary malformations of the sense organs in animals develop shortly after birth, though in stages which in man occur before birth. It thus appears that degeneration and necrosis of previously well formed parts may occur at any age; their significance during embryonic life is just beginning to be appreciated.

EXPERIMENTAL OBSERVATIONS ON THE RELATION OF INCREASED INTRACRANIAL PRESSURE AND PULMONARY EDEMA. William Harrison (by invitation) and Averill A. Liebow, New Haven, Conn.

*Abstract.* It has long been known that severe pulmonary edema is usually found in patients who die following a large and rapid increase of intracranial pressure. The present investigation was designed to determine the mechanisms involved. Access to the lesser circulation in dogs was obtained by attaching modified London cannulas to the pulmonary artery and left auricle at a prior operation. At the time of the experiment pressures in these structures as well as those in the aorta, femoral vein, and subarachnoid space were recorded simultaneously with a bank of Hamilton manometers. The thorax was by then well healed and kept closed during the recordings while the animals were under chloralose basal anesthesia. As the intracranial pressure was raised to levels approaching that of the original mean systemic arterial pressure, bradycardia usually appeared. Simultaneously, there was a rise in left auricular pressure (and therefore pulmonary capillary pressure) which in some instances, if the bradycardia was maintained, exceeded the oncotic pressure with consequent pulmonary edema. With escape from bradycardia these pressures returned to normal. Atropine prevented the bradycardia, and permitted a much greater rise in the intracranial pressure with an associated enormous systemic hypertension without immediate death in apnea. Ultimately, under these conditions, there was left-sided heart failure, and with it a rise in left auricular pressure, sometimes sufficient to result in pulmonary edema.

STRUCTURAL CHANGES PRODUCED IN BROWN-PEARCE CARCINOMA CELLS BY MEANS OF A SPECIFIC ANTIBODY. Bernard Kalfayan (by invitation) and John G. Kidd, New York, N.Y.

*Abstract.* Brown-Pearce carcinoma cells lose their viability when incubated with the antibody that reacts specifically with a distinctive constituent of them (J. Exper. *Med.*, 1946, 83, 227–250). Furthermore, contrary to previous observations, they also manifest characteristic structural changes, evident both in fresh and in fixed and stained preparations. When the carcinoma cells are suspended individually in a buffered Ringer's solution and incubated at 37° C. with rabbit serum containing the specific antibody and complement, their cytoplasm immediately begins to swell and the cells soon become spherical. Within 5 minutes vesicles, 1 or 2 $\mu$ in diameter, begin to appear in the cytoplasm, often at its periphery, and the cytoplasm begins to lose its affinity for basophilic dyes. By this time the cells have lost their viability, as transplantation tests show. As the cytoplasm continues to swell its basophilism diminishes rapidly, so that little remains after 15 minutes. Often at this stage a mantle comprised of small, amorphous, acidophilic granules can be seen around some of the cells. Soon thereafter, in the fixed and stained preparations, the cyto-

plasm appears empty except for a moderate amount of amorphous acidophilic material together with a few rod-like, granular, and vesicular bodies which are dispersed irregularly in it and often adhere to the wrinkled plasma membrane. In fresh cells examined with the phase-contrast microscope at this stage the formed materials appear to be suspended irregularly in a transparent fluid that distends the cytoplasm. "Blisters" do not develop from the plasma membranes of the antibody-treated cells, as they regularly do within a few minutes from the outer surfaces of viable cells suspended in saline solution or in rabbit serum devoid of antibody. The nuclei often remain intact for some time after the cytoplasmic changes have become advanced, although the nucleoli are generally inconspicuous in the antibody-treated cells. Within 30 to 60 minutes, however, the nuclei of some cells become moderately shrunken and the nuclear membranes wrinkled, though usually the nuclear sap remains transparent and the chromatin pattern unaltered for several hours.

It seems noteworthy that the antibody does not agglutinate the Brown-Pearce carcinoma cells during prolonged contact, and that it does not produce changes in the cells of the V-2 rabbit carcinoma or in those of normal rabbit kidney and liver parenchyma in control tests. Yet it induces the described changes in practically every Brown-Pearce carcinoma cell exposed to its action *in vitro*. The changes are not manifest in Brown-Pearce carcinoma cells treated with rabbit and guinea-pig sera devoid of the specific antibody. Furthermore, they have not been reproduced in Brown-Pearce carcinoma cells exposed to the action of various surface active agents and enzymes, and they differ notably from the alterations induced in these cells by means of hypotonic solutions or autolysis.

THE RELATIONSHIP OF THE PANCREAS TO THE ABSORPTION OF IRON. Thomas D. Kinney and (by invitation) Clement A. Finch, Nathan Kaufman, Mark Hegsted, and Phillip F. Partington, Cleveland, Ohio.

*Abstract.* Pancreatic ducts of dogs were ligated and divided under aseptic conditions. Care was taken not to disturb the common bile duct and the circulation of the duodenum and pancreas. The dogs were sacrificed at periods of 2 to 5 months. Those animals in which satisfactory ligation of the pancreatic ducts and marked atrophy of the pancreas could not be demonstrated by gross and microscopic examination were discarded. The livers were stained for iron and the amount of iron present graded quantitatively from 1 to 6 plus. Samples of liver were digested and iron analyses were done on aliquots of the digest using a buffer solution with thioglycolate as a reducing agent and orthophenanthroline as an indicator. In all animals there was agreement between histologic grading and chemical analyses. Group I consisted of control dogs in which the pancreatic ducts were not ligated and which were fed purina dog chow alone. The livers of these dogs were graded 1 to 2 plus on histologic examination for iron and the liver iron values varied between 3.0 and 31.0 mg. per 100 gm. of wet tissue. Group II was made up of dogs in which successful ligation of the pancreatic ducts was done and in which there was marked atrophy of the pancreas. The livers were graded 4 to 6 plus for iron and the liver iron values ranged from 54.25 to 122 mg. per 100 gm. of wet tissue. Iron was present in both parenchymal liver cells and Kupffer cells. Further, one dog, in which the pancreatic ducts were tied, was given $Fe^{59}$ by mouth. There was a marked increase in the amount of radio-iron present in the liver over the amount in the livers of non-operated controls. Group III consisted of dogs with successfully ligated pancreatic ducts, which were fed raw pancreas daily in addition to the purina dog chow. These livers were graded 1 to 3 plus and the liver iron values varied between 19.2 and 35.7 mg. per 100 gm. The results indicate that ligation of the pancreatic ducts in dogs is followed by increased absorption of iron from the gastro-intestinal tract with marked increase in liver iron values.

THE PATHOLOGY OF THE ENDOCRINE SYSTEM IN ALBRIGHT'S SYNDROME. H. Edward MacMahon, Boston, Mass.

*Abstract.* Recent advances in the study of pituitary-adrenal functions, coupled with startling therapeutic results, have initiated a flood of investigations and focused renewed interest on all syndromes in which these glands are particularly involved. Albright's syndrome or syndrome X, a recurring clinical entity characterized by (a) osteitis fibrosa disseminata, (b) areas of pigmentation, and (c) precocious puberty in females, is such an example, and the post-mortem findings in one of the cases included in the original group described by Albright and his associates form the basis of this study.

This patient, a female child, 10 years of age, died as the result of diffuse lobular pneumonia. She had been under observation since early infancy because of (a) widely disseminated bone lesions, (b) extensive patchy brown pigmentation of the skin, and (c) a very unusual menstrual history beginning at the age of 4 months. At the time of her death, her sexual characteristics resembled those of an adult. Follicle-stimulating hormone had been demonstrated in the blood and excess androgens and estrogens had been found in the urine. Blood pressure readings, basal metabolic rates, and blood sugar determinations had been consistently above normal. In addition to these, the child was mentally retarded. The endocrine system of this child has been very carefully studied and histologic changes have been found in each of the endocrine glands. The anterior lobe of the *pituitary body* was enlarged and nodular. It was very cellular and contained many atypical forms including scattered Crooke cells. Mitotic figures were found in both chromophobes and chromophils but proliferation was most active in the basophil cells. The *adrenals* were larger than normal and showed a nodular hyperplasia of both reticular and fascicular zones. There was herniation of the latter well out into the pericapsular fat. The cortical cells were rich in lipid; those of the medulla were unchanged. The *thyroid gland* showed nodular enlargement, follicular hyperplasia, and marked diminution in colloid. The *islets* of the pancreas were numerous, large, unusually cellular, and very poorly confined. The *parathyroid glands* were larger and more cellular than normal, and in each there was an abundance of large water-clear cells. The *pineal body* was slightly enlarged and composed almost exclusively of nonfibrillar parenchymatous cells. The *ovaries* were larger than normal for this age and the surfaces were smooth and lobulated. All sections revealed many developing and regressing follicles but there was no evidence of ovulation or corpus luteum formation. Small collections of androgen-positive cells were found in the hilus of each ovary.

In summary, hypertrophy and hyperplasia, consistent with increased functional activity, was common to each of the endocrine glands, a finding anticipated by the clinical signs and symptoms. The most striking changes were found in the pituitary body. In the light of our present knowledge it would appear that this gland, as had been postulated clinically, had been the dominating force responsible for the complex polyglandular dyscrasia which had been such an important component in the over-all clinical syndrome.

THE RELATIONSHIP OF COAGULASE-GLOBULIN TO PROTHROMBIN AS STUDIED BY THE STAPHYLOCOAGULASE REACTION. John B. Miale (by invitation), Marshfield, Wis.

*Abstract.* Some strains of staphylococci produce a substance which clots oxalated or citrated plasma. When decreasing concentrations of staphylocoagulase react with human plasma, the curve of the clotting times is hyperbolic. Staphylocoagulase is by itself unable to clot fibrinogen of the highest purity (fraction 1-2A), and the clotting of fibrinogen results only when staphylocoagulase reacts with a plasma globulin factor, coagulase-globulin, to form a new product, coagulase-thrombin, which promptly produces clotting of fibrinogen. Furthermore, the mechanics of

the production of coagulase-thrombin also were shown to produce a hyperbolic curve. The similarity of this reaction to the activation of prothrombin by calcium and thromboplastin suggested that staphylocoagulase might be an enzyme which activated prothrombin. However, the reaction takes place in the absence of calcium and in the presence of certain azo dyes and with heparin.

Human oxalated plasma can be made prothrombin free (both by the one stage and two stage tests) by storage and adsorption with $Ca_3(PO_4)_2$, $Al(OH_3)$, $BaSO_4$, and $Mg(OH)_2$. Plasma which has been stored, but otherwise untreated, is clotted normally by staphylocoagulase, but after adsorption with the above substances it was unclottable by staphylocoagulase. Plasma adsorbed with $CaF_2$ is, however, clottable by staphylocoagulase and also shows no prothrombin activity. The same characteristics are shown by plasma filtered five times through a Seitz filter, providing certain requirements of plasma volume to asbestos mass are met. Plasmas adsorbed on $CaF_2$ or asbestos are, therefore, assumed to be prothrombin free but to have an unreduced titer of coagulase-globulin. Prothrombin free plasma, defibrinated with minimal amounts of thrombin and then precipitated with $(NH_4)_2SO_4$ or by the Mellanby technic, yields precipitates of very high coagulase-globulin titer but containing no demonstrable prothrombin activity. Furthermore, I have been able to demonstrate only a very slight prothrombin accelerator effect with coagulase-globulin products, and this is probably due to minimal contamination with this globulin which has somewhat similar chemical properties.

In view of these findings, it seems justified to assign a definite function to coagulase-globulin in the blood coagulation scheme. Our studies to date support this working hypothesis, that coagulase-globulin is converted to coagulase-thrombin by staphylocoagulase and other, as yet unknown, factors and that this coagulase-thrombin acts directly on fibrinogen and also on prothrombin, not in the same way as thromboplastin plus calcium plus cephalin, but catalytically, as is done by the thrombin produced by "classical" prothrombin.

THE TISSUE ORIGIN OF NEOPLASMS. STUDIES ON THE EARLY PHASES OF INDUCED FIBROSARCOMA IN THE HAMSTER. Anderson Nettleship, Little Rock, Ark.

*Abstract.* Until sufficient data are accumulated on the earliest stages of neoplasms, understanding of the intrinsic mechanism of new growth remains incomplete. Fibrosarcomas induced by 20-methylcholanthrene were found to develop rapidly in golden hamsters. Because of early and rapid growth, this was considered an ideal situation in which to study neoplasm inception. Animals were injected with 20-methylcholanthrene in olive oil or saline suspension. In order to mark the site of injection the solutions contained carbon black. Animals were sacrificed and the sites studied at 24, 48, and 72 hours; 4, 5, 6, 10, 14, 21, 28, and 35 days; 7 weeks; and when tumors appeared at 68 days. Adequate controls were studied on paralleling dates.

Within 24 hours there is marked edema of the connective tissue. Connective tissue cells are found distinctly different from others. These are detached cells which are marked by swollen, granular, pink-stained cytoplasm and a nucleus with fine, granular karyoplasm and marked nuclear membrane. These cells are broad and often tailed. They differ from macrophages which contain carbon pigment. They are not capillary endothelial cells. They appear to be derived from connective tissue cells. These unique cells persist throughout all phases prior to the actual growth of the sarcoma and it is considered that it is from them that the actual sarcoma arises. Even at 48 hours the fibroblasts have formed sheets of cells which are loose and edematous. By 4 days a scattering of inflammatory cells is present, chiefly lymphocytes and monocytes. The loose edematous sheets have been replaced by tight loci of cells; the marked fibroblastic changes in the cells continue. At the end of the first week the usual staining properties are replaced by marked stainability of the connective tissue cells. The process continues with continued growth activity

but few mitotic figures up to the 7th week when they become more numerous. The transition is not sharp. Control groups show fibroblastic proliferation and some macrophages filled with carbon. The individual fibroblasts, as described, do not appear in the controls. Special stains confirm the fibroblastic nature of the presarcomatous cells. The morphologic alteration of connective tissue cells into free cells with distinctive characteristics persists until establishment of sarcoma is noted. The cells are altered within 24 hours of injection of the carcinogen. The changes from these cells into frank sarcoma are smooth and gradual, taking place during an 8-week period. They are marked by local proliferation of changed connective tissue fibroblasts into groups, loci, and sheets, and finally sarcoma. Injection of the carcinogen in oil produces little cell necrosis in contrast to the saline suspension of methylcholanthrene in which there is widespread cellular necrosis and giant cell formation.

TISSUE CHANGES INDUCED BY DESOXYCORTICOSTERONE ACETATE (DCA) IN THE GUINEA-PIG, WITH PARTICULAR REGARD TO CONNECTIVE TISSUE. Conrad L. Pirani and (by invitation) Robert C. Stepto, Chicago, Ill.

*Abstract.* Previous studies in our laboratory have shown that the administration of large amounts of desoxycorticosterone acetate (DCA) in guinea-pigs causes nephrosclerosis with marked tubular hypertrophy, slight to moderate arteriosclerosis, and hypertrophy of the heart. Occasionally, small perivascular foci of histiocytes and mononuclears were seen in the heart. Although no lesions of the "rheumatic type" could be produced by this method, either in the cardiovascular system or in the joints, these studies were extended by investigating the effect of DCA on mesenchymal tissue in healing laparotomy wounds. Operations were performed on 44 guinea-pigs under nembutal anesthesia. They were sacrificed at regular intervals 1 to 14 days after operation. Daily injections of 3 mg. of DCA in sesame oil were made subcutaneously for 5 days prior to operation and continued throughout the experiment. The control animals received daily injections of sesame oil for the same period. Blocks taken from the center of the excised wounds were fixed in 10 per cent formalin and by freezing-drying with the Altmann-Gersch technic. Sections were stained with hematoxylin and eosin, van Gieson, Foot (reticulum), toluidin blue, and by the Hotchkiss-McManus periodic acid routine. The results indicated that DCA induces the production of a greater amount of granulation tissue by stimulating the proliferation of fibroblasts and the formation of an excessive amount of ground substance. There was, however, a slight to moderate lag in the maturation process of both cellular and intercellular elements. This phenomenon, which was particularly evident on the tenth postoperative day, did not appear to be mediated through changes in blood proteins or ascorbic acid. The presence of changes in granulation tissue and their lack in resting connective tissue of DCA-treated guinea-pigs confirmed the view that a profound difference in the response mechanism exists between resting and actively proliferating connective tissue.

PERIARTERITIS NODOSA OF THE APPENDIX. William R. Platt, Philadelphia, Pa.

*Abstract.* Periarteritis nodosa is characterized by variegated protein manifestations among which abdominal signs and symptoms are prominent clinical features. In the 5 cases described by the author these abdominal symptoms were severe enough to convince the examining physician that an acute surgical condition existed. Further confirmation was obtained in 3 of these cases, in 2 by necropsy examination and in one by biopsy of the gastrocnemius muscle. An additional diagnostic procedure, that is, the recently developed "freezing serum" test, is described as another laboratory aid in the possible earlier preoperative and ante-mortem detection of this collagen disease entity.

AN EVALUATION OF THE CYTOLOGIC TECHNIC IN THE RECOGNITION OF MALIGNANT UTERINE NEOPLASMS. James W. Reagan (by invitation) and R. T. Schmidt (by invitation), Cleveland, Ohio.

*Abstract.* Employing simultaneous cervical scraping and aspiration of the cervical canal, a total of 3,000 slides from 1,000 consecutive gynecologic surgical cases were studied objectively and the results were compared with the histopathologic findings. Of 72 specimens from patients with proved squamous cell carcinoma, cytologic recognition was possible in 71; while of 34 specimens from patients with adenocarcinoma, exfoliated cells were recognized in only 30. One patient with coexisting carcinoma and sarcoma was examined on two occasions and in both instances cytologic recognition was possible. A total of 6 specimens had indecisive pathologic diagnoses and were interpreted as containing malignant tumor cells by cytologic methods; these are considered unproved or incorrect interpretations. Of the remaining 886 specimens neither the cytologic technic nor the histopathologic findings offered evidence of malignant uterine neoplasm although the nature of the material was such that carcinoma was not excluded in many instances. The cytologic findings were compared with the histopathologic diagnoses on endometrial curettings and cervical biopsies. An evaluation of the method demonstrates that of the 55 cases of cervical squamous cell carcinoma seen during a period of 9 months, 8, or 14.5 per cent, were recognized solely through the use of the cytologic technic.

PLASMACYTOSIS OBSERVED POST MORTEM IN CASES EXHIBITING HYPERGLOBULINEMIA OR SIGNS OF HYPERSENSITIVITY. Theodore Robertson (by invitation), New York, N.Y.

*Abstract.* Plasmacytosis is produced readily in animals by hyperimmunizing with various antigens. As other workers have shown, the lymph nodes, omentum, renal pelvic fat, spleen, and bone marrow all participate in an increased production of plasma cells. This plasmacytosis has been related to the production of antibody globulins. Clinical reports indicate that an increase in plasma cells of the sternal marrow has been found more or less regularly in association with hyperglobulinemia in serum sickness, although plasmacytes have not always been conspicuous in the marrow of patients exhibiting hyperglobulinemia as a manifestation of other diseases. The literature contains but few reports of cases of plasmacytosis examined post mortem.

Abnormal accumulations of plasma cells unrelated to known infectious processes have been found in 11 of the last 1,500 necropsies performed in this department. In 2 cases of death associated with hypersensitivity to sulfadiazine (*Am. J. Med.*, 1950, in press), hyperglobulinemia was present and there was extensive plasmacytic infiltration of many organs, in addition to the well recognized lesions of sulfonamide hypersensitivity. Four other cases of presumed drug and transfusion hypersensitivity have also exhibited plasmacytosis. One of these had, in addition, renal lesions similar to those of disseminated lupus erythematosus; another, interstitial nephritis; and another, an acute necrotizing arteritis. Plasmacytosis and hyperglobulinemia were present in each of 4 cases of disseminated lupus and in 1 of 3 cases of polyarteritis nodosa.

Plasmacytosis was most evident in the lymph nodes. The plasma cells regularly predominated in the medullary cords of nodes throughout the body and not infrequently in the cortex also. A transition was traced from primitive reticular cells to typical Marshalko plasma cells. In a case of sulfonamide hypersensitivity in which the serum globulin was 11.2 gm. per cent, the lymphoid tissues everywhere produced plasmacytes almost exclusively. The spleen was the next most frequent site of plasma cell proliferation and giant forms were often evident. Plasmacytosis was common, but less apparent, in the bone marrow. The adventitia of the renal pelvis often contained plasmacytes, while accumulation of these cells in the cortex of the

kidney, about small blood vessels, in the pericardium, and in the liver were found only in severe cases. The amount of plasmacytosis in the various cases roughly paralleled the hyperglobulinemia. Plasmacytosis has been found also in each of 4 additional cases of lupus and in one of 3 additional cases of polyarteritis subsequently examined, and in 2 cases of allergic asthma. In all, 18 cases of plasmacytosis have been observed. Hyperglobulinemia was present in 10 of 11 cases in which serum globulins were determined late in the course of the disease. In all cases exhibiting plasmacytosis, except those of lupus, there was a history indicating hypersensitivity, *e.g.*, asthma, hay fever, or cutaneous or febrile reactions to drugs or transfusions.

MORPHOLOGIC CRITERIA OF EXFOLIATED MALIGNANT CELLS. Milton Rosenthal (by invitation), San Francisco, Calif.

*Abstract.* The continuing study of exfoliated malignant cells shows that the diagnostic value of isolated, smeared out cells is due to the lack of shrinkage artefacts which are commonplace in fixed tissue. It is possible to detect and differentiate false hyperchromatism due to overstaining and shrinkage of nuclei from true chromatin increase. The same is true of malignant nuclear irregularities and shrinkage wrinkling. The spreading out of whole cells allows a better evaluation of such malignant properties as increase and enlargement of nucleoli and karyosomes, increase in the nucleocytoplasmic ratio, hypertrophy of malignant cells over their benign counterparts in cell type and maturation, and cell crowding with mutual nuclear distortions. The isolation of cells permits a nicer evaluation of the degree of cornification because of the increased optical index of refraction of the cytoplasm which causes interference phenomena at the interface between the cell borders and mounting medium. Nucleoli of moderate size may be normal in cells with mild cornification, but indicate malignancy in cells of advanced differentiation. The detection of cornification allows a differentiation of small hypercornified malignant cells of epidermoid carcinoma from histiocytic cells with similar nuclear predominance and hyperchromatism.

The morphologic aberrations of malignant cells are generally familiar, but their degree of reliability and quantitative evaluation are increasingly delimited by continuing empirical association with subsequent proof of carcinoma by biopsy, and their absence from material obtained from patients with non-malignant disease. Direct smears of fresh tissue, both malignant and inflammatory, aid in gaining experience with the upper limits of aberrations which may resemble malignant properties in non-malignant atypical cells. The following outline includes the cytologic aberrations which have been found useful in diagnosis or presumption of malignancy, although each "criterion" of malignancy must be evaluated in degree, differentiated from technical artefacts, and a borderline established above which bizarre non-malignant cells do not cause misinterpretation. Many of the criteria must be further qualified in respect to cell type and degree of maturation.

A. Cell size
   1. Cell (nuclear) hypertrophy (over the non-malignant counterpart, with respect to type of cell and degree of maturation)
   2. Variation in size
B. Nuclear predominance (increase in nucleo-cytoplasmic ratio)
C. Irregularity of nuclear contour
   1. Jagged irregularity
   2. Multilobulation and scalloping (in tumor giant cells)
D. Hyperchromatism
   1. Complete opacity
   2. Thickened nuclear rims
   3. Increased, enlarged, and irregular chromatin knots
   4. Massive chromatin condensations
E. Nucleolar increase
   1. Massive nucleolus
   2. Multiple prominent nucleoli
   3. Irregularity of nucleoli

F. Cell groups
   1. Discrepancy in maturation (juxtaposition of undifferentiated cells to highly differentiated cells)
   2. "Crowding" (especially with mutual nuclear distortions)
   3. "Clumping" or "molding"
   4. "Cannibalism"
G. Abnormal mitotic figures
H. Special malignant cell types
   1. Malignant acini (adenocarcinoma)
   2. Huge cytoplasmic vacuole (adenocarcinoma)
   3. Tiny malignant cells
   4. Spindle cells
   5. Tumor giant cells
   6. Undifferentiated malignant cells (*i.e.*, with germinal cell chromatin pattern)
   7. Differentiated malignant squamous cells
      a. Pavement cell type
      b. Hypercornified type
      c. Malignant squames

ABSCESSES IN THE VALVE RINGS OF THE HEART, A FREQUENT AND NOT WELL RECOGNIZED COMPLICATION OF ACUTE BACTERIAL ENDOCARDITIS. Walter H. Sheldon and Abner Golden, Atlanta, Ga.

*Abstract.* Since 1947 we have observed 10 patients with acute bacterial endocarditis who at autopsy showed one or more abscesses in the valve rings. These abscesses measured from one to several centimeters in size, and involved most commonly the aortic valve ring, although the other rings were not spared. Histologically the abscesses consisted of necrotic centers surrounded by granulation tissue. One or multiple points of rupture into the heart chambers were present in some of the lesions in which laminated blood clot was often found near the perforation. Except for variations in location and age, the abscesses were strikingly similar in all cases. The infection was by pneumococci in 9 patients and by *Staphylococcus aureus* in one. All but one patient had received penicillin over periods ranging from 3 to 27 days in total amounts of from 600,000 to 69,000,000 units. The endocarditis involved most frequently the aortic valve, followed in order by the mitral, tricuspid, and pulmonic valves. Pneumonia, meningitis, or both had been present on admission in 8 patients. At autopsy the treated cases showed advanced or complete healing of these lesions. Histologic study of the valvular lesions also revealed varying and often advanced repair.

The anatomical relationships of the heart explain the involvement of more than one valve by the abscesses, but the pathogenesis of the abscesses is uncertain. An embolic origin is suggested by the finding of occluded vessels similar to those which are the basis of septic embolic abscesses elsewhere. The laminated thrombi in some of the lesions suggested that they might have originated from mycotic aneurysms of the vessels in the valve ring. Increased vascularity of this region subsequent to rheumatic heart disease, arteriosclerosis, or cardiovascular syphilis might represent a predisposing factor. In this connection it may be significant that 6 of our patients had syphilitic aortitis and one other patient showed calcification of the mitral ring. It is our impression that these abscesses are now seen more frequently. The same factors, which prevented the healing of bacterial endocarditis prior to antibiotic therapy, may account for the progression of the abscesses. It is possible that therapeutic concentrations of penicillin do not suffice to penetrate valve ring abscesses, which can then act as sources of persistent infection.

EXPERIMENTAL CALCINOSIS ON A NUTRITIONAL BASIS. Paul B. Szanto, Dorothy Nelson (by invitation), J.P. Weinmann (by invitation), A. C. Ivy (by invitation), and Hans Popper, Chicago, Ill.

*Abstract.* Morphologic changes occurred in guinea-pigs as a result of prolonged administration of diets, the pertinent features of which were high calcium and especially high phosphorus content, normal content of vitamin D and C, high con-

tent of protein, low content of fat, and various amounts of cholesterol. All diets promoted good growth. Calcium deposits were observed in various locations in animals receiving these diets 8 months or longer. When nodules became palpable, the guinea-pigs were losing weight and became inactive. X-ray pictures revealed calcium deposits in the skin, about the joints, in the intercostal and abdominal muscles, and along the vertebral column. The more consistent biochemical findings were elevation of serum phosphorus and total cholesterol; calcium was only slightly elevated and the alkaline phosphatase was normal. The animals revealed fatty changes and uniform nodularity of the liver, granular atrophy of the kidneys, calcified plaques in the aorta, and calcified nodules in the locations indicated by the x-ray examination.

Histologically the cutaneous nodules consisted of deposits of calcium phosphate and extracellular cholesterol. In the kidneys, the Bowman's spaces and the convoluted tubules were dilated, the epithelial cells were calcified and the lumina of the tubules contained calcium deposits. Histochemically, a marked decrease of alkaline phosphatase in the renal cortex was noted. Calcium deposits were observed in the mucosa and submucosa of the stomach, and in the zona reticularis of the adrenals. The liver showed fatty metamorphosis, portal fibrosis with altered reconstruction, and calcification of scattered liver cells. The aorta showed destruction of the elastica in the media with replacement by fibroblasts, calcification of the internal elastic membrane and of the media, thickening of the intima, and frequently occlusion of the vasa vasorum by lipophages. The coronary arteries were occluded by lipophages with resulting myocardial necrosis. The skeleton showed resorption of lamellated bone, compensatory bone formation in the form of osteophytes on the periosteal surfaces, and replacement of the fatty marrow by fibrous tissue. Calcification with surrounding ossification in the periodontal membrane was noted.

The calcium deposits in the cutaneous and muscle tissues resemble the findings in calcinosis universalis in the human. The bone findings simulate those in hyperparathyroidism. The combination of universal calcinosis, vascular changes, and bone lesions of hyperparathyroid character has been described in human beings without established etiology. The experimental condition on a nutritional basis results, in addition, in fatty cirrhosis.

SARCOMATOID GROWTHS RESULTING FROM MAMMARY CARCINOMA CELLS THAT HAD SOJOURNED IN IMMUNE MICE. Helene Wallace Toolan (by invitation) and John G. Kidd, New York, N.Y.

*Abstract.* Tumors often result when the cells of a transplantable $C_3H$ mammary carcinoma are transferred back to susceptible $C_3H$ hosts after several days' sojourn in the subcutaneous tissues of A mice immune to them (*Federation Proc.*, 1949, **8**, 360; 373). The growths, however, are not mammary carcinomas, but fibrosarcomas, as judged histologically. This has proved true in 13 of 15 experiments in which the carcinoma cells remained 4 days or more in the immune mice; in no instance has an ordinary mammary carcinoma been obtained from cells that had lain as long as 6 days in the subcutaneous tissues of immune hosts. The sarcomatoid growths are comprised of spindle-shaped cells with elongated nuclei and moderate amounts of cytoplasm that stains lightly with basophilic dyes; the cells often lie whorled in parallel bundles amidst profuse and interlacing bands of collagen. The growths differ notably from the transplantable mammary carcinoma, which is made up of polyhedral or slightly elongated elements with spherical nuclei and an abundance of basophilic cytoplasm. These cells regularly arrange themselves into solid cords and pseudopapillary masses, to form a carcinoma simplex alveolare according to Apolant's classification. In regions where the tumor cells are proliferating as individuals, they are often considerably elongated and tend to be arranged more loosely among the compact syncytial masses, and in poorly nourished areas the cells of the carcinoma may be somewhat fusiform; but in neither case is collagen or reticulin associated.

One of the sarcomatoid growths, derived from mammary carcinoma cells that had lain 5 days in an immune A host, has now been transplanted during 18 months (25 tumor-generations) in $C_3H$ animals. Like all of the sarcomatoid tumors previously mentioned, it grows more slowly than does the transplanted mammary carcinoma. Originally composed of spindle-shaped cells arranged in whorls amid much collagen, after several transfers it took on the appearance of an atypical anaplastic carcinoma with more rounded cells. The growth is still, however, unlike the transplanted mammary carcinoma from which it took origin; its cells do not arrange themselves in syncytial masses, and moderate amounts of reticulin and collagen are associated with them. The cells in each tumor-generation have been remarkably uniform.

Sarcomatoid growths have frequently been reported heretofore in association with spontaneous and transplanted mammary carcinomas of mice. The majority of workers have considered that they result from the malignant transformation of stroma, though others have viewed the spindle-shaped elements as variants of the carcinoma cells. Our findings support the latter hypothesis. It is noteworthy that the sarcomatoid transformation has not heretofore been induced. Further work will be required to learn whether the induced alteration in form and behavior of the mammary carcinoma cells is conditioned by the immune state of the temporary host or by adverse conditions that can be duplicated in the absence of immunity.

APPENDICEAL ARTERITIS. Tobias Weinberg, Baltimore, Md.

*Abstract.* An analysis is made of a series of 54 cases of appendiceal arteritis found in the routine examination of surgically removed appendices. The incidence in routine surgical material as well as in a consecutive series of autopsies is noted. The striking sex linkage and the age incidence are analyzed. The clinical aspects are only briefly touched upon inasmuch as this aspect will be the subject of a subsequent report. The histopathologic nature of the lesion has been studied by both routine staining methods and by the use of special stains and technics.

FURTHER OBSERVATIONS ON COLOR REACTIONS WITH SERA OF PATIENTS WITH MALIGNANT NEOPLASMS. Emil Weiss, Chicago, Ill.

*Abstract.* A modification of the original procedure of color reactions with malignant sera has been devised. It is a combination of a color reaction and a precipitation reaction. Three dyes are used: indoin blue, Janus black, and Nicholson's blue. The dyes are dissolved in isopropyl alcohol 1:1000, as stock solutions. For the test the dyes are further diluted 1:20. As a diluent a 35 per cent dilution of isopropyl alcohol containing a small amount of lipoids extracted from dried beef heart (0.5 mg. of beef heart is added to 1 cc. of 35 per cent isopropyl alcohol, kept 2 days at room temperature and then filtered) is used. The blood should be taken before breakfast. Turbid, hemolytic, or icteric sera should not be used. Each unknown serum requires 3 tubes, 1 for each dye. The first tube receives 0.95 cc. of indoin blue, the second tube 0.95 cc. of Janus black, and the third tube 0.95 cc. of Nicholson's blue. All 3 tubes receive 1 drop of the unknown serum. Each dye has a positive control containing 0.95 cc. of the dye and 1 drop of a positive serum; the negative control contains 0.95 cc. of the dye and 1 drop of the negative serum. The tubes are shaken for a few seconds and then the immediate reactions are read. A positive tube becomes rapidly turbid, and shows flocculation within 30 minutes, and, after 12 hours, a dark colored sediment and a light supernatant fluid. A negative tube becomes only slightly turbid or opalescent, and remains the same throughout the test. The procedure is positive in 90 per cent of patients with malignant neoplasms, and gives 10 per cent non-specific reactions. The test applies to all types of cancer.